SURVEILLANCE OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AMONG PATHOGENS ISOLATED IN PUBLIC SECTOR HOSPITALS ASSOCIATED WITH ACADEMIC INSTITUTIONS IN SOUTH AFRICA

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Declaration

I, Peter S. Nyasulu, declare that this thesis is my own original work. It is being submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. It has not been submitted to any other University before for any examination or degree.

Peter Suwirakwenda Nyasulu   30th November, 2014

Dedication

I dedicate this work to my loving parents Rodwell Suwirakwenda Nyasulu (my dad), Weston & Elsie Nkowani, (my uncle and auntie), who were all instrumental in my upbringing since the age of 1 year and sacrificed their resources for the cause of bringing education near to me. Sadly, they all passed on while I was pursuing this Degree.

Publications and Presentations arising from the Thesis

Original papers

1. **Peter S Nyasulu,** Christine Praszko, Nontombi Mbelle. A Narrative Review of the Laboratory Information System and Its role in Antimicrobial Resistance Surveillance in South Africa. *Advances in Microbiology*, Volume 4, Issue 10, pages 692-696, August 2014.

2. **Nyasulu P,** Kasubi M, Boniface R, Murray J. Understanding laboratory methods and their impact on antimicrobial resistance surveillance, at Muhimbili National Hospital, Dar es Salaam, Tanzania. *Advances in Microbiology*, Volume 4, Issue 1, pages 33-38, January 2014.

3. **Nyasulu P,** Murray J, Perovic O, Koornhof H. Antimicrobial resistance among selected nosocomial pathogens in South Africa: Systematic review of published literature. *Journal of Experimental and Clinical Medicine,* Volume 4, Issue 1, Pages 8-13, January, 2012.

4. **Nyasulu P**, Cohen C, de Gouviea L, Feldman C, Klugman KP, von Gottberg A. Increased risk of death in HIV-Infected children with pneumococcal meningitis in South Africa, 2003-2005. *Pediatric Infectious Disease Journal;* Volume 30, Issue 12, Pages 1075-1080, July, 2011.

Published abstracts and conference presentations

- **Nyasulu P**, Perovic O Murray J, Manda S, Koornhof H. Antimicrobial resistance among clinical isolates of invasive *Staphylococcus aureus* from seven academic hospitals in South Africa over a 12 months period. 4th Infection Control Africa Network (ICAN), Cape Town, 26-29th November, 2012.

- **Nyasulu P**, Perovic O, Murray J, Luchters S, Chasela C, Koornhof H. Trends and Pattern of Antimicrobial Resistance among Blood Culture Isolates of Selected Bacterial Pathogens in

South Africa, 2005-2009.15[th] International Congress of Infectious Diseases, Centara Grand, Bangkok, Thailand 13th-16th June, 2012.

- **Nyasulu P**, Murray J, Perovic O, Koornhof H. Antimicrobial resistance among selected nosocomial pathogens in South African hospitals: Systematic review of published literature. 7th Public Health Association of South Africa conference, Sandton, Johannesburg 28th -30th November, 2011.

- **Nyasulu P,** Murray J, Perovic O, Koornhof H. Changing the Landscape of Antimicrobial resistance among nosocomial pathogens in South Africa: Systematic review of published literature.1st Global Forum for Bacterial Infection. New Dehli, India, Oct 3-5, 2011.

- **P. Nyasulu**, C. Cohen, A.von Gottberg, L. de Gouveia, V. Quan, C. Feldman, K. P Klugman. Potential predictors of death in children with Invasive Pneumococcal Disease in South Africa, 2003-2005. Research dissemination day, School of Public Health, University of the Witwatersrand, 14th May 2009.

- **P. Nyasulu,** C. Cohen, A. von Gottberg, L. de Gouveia, V. Quan, C. Feldman, K. P. Klugman. Increased Risk of Death in HIV-Infected Patients with Pneumococcal Meningitis, South Africa, 2003–2005. *International Journal of Infectious Diseases*. Dec 2008 Vol. 12, Pages e44-e62. 13th International Congress of Infectious Diseases, Kuala Lumpur Convention Centre, Kuala Lumpur, Malaysia, 12th-14th June, 2008.

Comprehensive summary

Background: Antimicrobial resistance (AMR) is a global public health challenge since infection with resistant organisms may cause death, can spread across the community, and increase health care costs at individual, community and government level as more expensive antimicrobials will have to be made available for the treatment of infections caused by resistant bacteria. This calls for urgent and consolidated efforts in order to effectively curb this growing crisis, to prevent the world from slipping back to the pre-antibiotic era. The World Health Organization made a call in 2011 advocating for strengthening of surveillance and laboratory capacity as one-way of detecting and monitoring trends and patterns of emerging AMR. Knowledge of AMR guides clinical decisions regarding choice of antimicrobial therapy, during an episode of bacteraemia and forms the basis of key strategies in containing the spread of resistant bacteria. The current study focused on *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), and *Pseudomonas aeruginosa* (PA), as they are common hospital acquired infections which are prone to developing resistance to multiple antibiotics.

Aim: The aim of this project was to assess and utilize the laboratory information system (LIS) at the National Health Laboratory Services (NHLS), as a tool for reporting AMR and monitoring resistance patterns and trends over time of clinical isolates of SA, KP and PA, cultured from the blood of patients admitted to seven tertiary public hospitals in three provinces in South Africa.

Methods: A retrospective and prospective analysis was done on isolates of SA, KP, PA from blood specimens collected from patients with bacteraemia and submitted to diagnostic microbiology laboratories of the NHLS at seven tertiary public hospitals in three provinces in South Africa. These hospitals comprised the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwanath Hospital (CBH), Helen Joseph Hospital (HJH), Steve Biko Pretoria Academic Hospital

(SBPAH), Groote Schuur Hospital (GSH), Tygerberg Hospital (TH) and the Universitas Hospital of the Free State (UH). For retrospective analysis, data submitted during the period July 2005 to December 2009 were used and for prospective analysis, data relating to AMR in SA, KP, PA, collected by the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa, (GERMS-SA) from July 2010 to June 2011 were used. AMR in these three pathogens to commonly used antimicrobial drugs was systematically investigated. Multivariate logistic regressions models were used to assess factors associated with AMR. In addition, a systematic review of research done to date on AMR in bacterial pathogens commonly associated with hospital-acquired infections was conducted in order to understand the existing antimicrobial surveillance systems and baseline resistance patterns in South Africa.

Results: A total of 9969 isolates were reported from the retrospective dataset. These were 3942 (39.5%) SA, 4466 (44.8%) KP and 1561 (15.7%) PA. From the prospective dataset, a total of 3026 isolates were reported, 1494 (49.4%) SA and 1532 (50.6%) KP isolates respectively. The proportion of invasive bacteraemia was higher in the <5 year old children. Nearly all strains of SA in South Africa were resistant to penicillin, and >30% up to as high as 80% were resistant to methicillin-related drugs among~560 invasive SA isolates over the two year period. Methicillin resistant *Staphylococcus aureus* (MRSA) rates significantly differed between hospitals (p=<0.001). The proportion of MRSA isolates in relation to methicillin-susceptible strains showed a declining trend from 22.2% in 2005 to 10.5% in 2009 (p=0.042). Emerging resistance was observed for vancomycin: 1 isolate was identified in 2006 and 9 isolates between July 2010-June 2011, and all except 1 were from Gauteng hospitals. The study found increasing rates of carbapenem-resisant KP of 0.4% in 2005 to 4.0% in 2011 for imipenem. The mean rate of extended spectrum beta lactamase (ESBL-KP) producing KP was 74.2%, with the lowest rate of 62.4% in SBPAH and the highest rate of 81.3% in UH, showing a significant geographical variation in rates of resistance (p=0.021). PA showed a tendency for multi-drug resistance with resistance rates of >20% to extended spectrum cephalosporins, fluoroquinolones and aminoglycosides

respectively. Emerging resistance in PA isolates was observed to colistin, showing a resistance rate of 1.9% over the 5 years period. In the multivariate model, age <5 years, male gender, and hospital location were factors significantly associated with MRSA, while ESBL-KP was significantly associated with age <5 years and hospital location.

Concluding remarks: The study has clearly demonstrated that AMR is relatively common in South Africa among children <5 years. Enhancement of continued surveillance of nosocomial infections through use of routine laboratory data should be reinforced as this will facilitate effective interpretation and mapping of trends and patterns of AMR. Therefore, the LIS as a tool for gathering such data should be strengthened to provide reliable AMR data for improved understanding of the extent of the AMR, and present evidence on which future policies and practices aimed at containing AMR could be based.

Key words: Laboratory information system, Trends, Patterns, Antimicrobial resistance, Bacterial pathogens, Nosocomial infections, Surveillance, Bacteraemia, Blood culture.

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Definition of terms

American Type Culture Collection (ATCC) is a biological resource center that focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other materials for research in the life sciences.

DISA is an orchid, the flower. It is a symbol and name of the laboratory information management system (LIMS) used by the National Health Laboratory Service (NHLS). The software was developed by the Ifocus Systec Company (Bangalore, India).

JEEVA is an integrated hospital management information system (HMIS) application through which all hospital functions are run. These include patients' registration, laboratory, pharmacy etc.

Laboratory information system (LIS) is a series of computer programs that process, store and manage data from all stages of medical processes and tests.

Laboratory information management system (LIMS) sometimes referred to as Laboratory information system (LIS) is a software-based laboratory and information management system that offers a set of key features that support a modern laboratory operations and is used interchangeably with LIS.

Nosocomial infection can be defined as infection occurring after 48 hours of hospital admission, 3 days after discharge or 30 days after an operation.

SENTRY is a program of antimicrobial resistance surveillance for Asia-Pacific regions and South Africa.

WHONET is free Windows-based database software developed by World Health Organization for the management and analysis of microbiology laboratory data with a special focus on the analysis of antimicrobial susceptibility test results.

TrakCare Lab is a laboratory information management system (LIMS) that offers accurate laboratory results reporting, improved laboratory efficiency and better business management.

List of Abbreviations

AMR	Antimicrobial resistance
AMRS	Antimicrobial resistance surveillance
APACHE	Acute physiology, age, chronic health evaluation
API	Application Programming Interface
ARPAC	Antibiotic Resistance Prevention and Control
ARSR	Antimicrobial Resistance Surveillance and Research
AST	Antimicrobial susceptibility test
BA-3D	BacT/Alert 3D
BAL	Bronchial Alveolar Lavage
BC	Blood Culture
CBSN	Canadian Bacterial Surveillance Network
CCDSS	Computerized Clinical Decision Support Systems
CDC	Centres for Disease Control and Prevention
CCSA	Collaborating Centre for Surveillance of Antimicrobials
CDW	Corporate Data Warehouse
CHB	Chris Hani Baragwanath Hospital
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CSF	Cerebral Spinal Fluid
DISA	Digital Information Systems Agency
EARS-Net	European Antimicrobial Resistance Surveillance Network
EC	*Escherichia coli*
ESBLs	Extended spectrum beta-lactamases
ESBL-KP	Extended spectrum beta-lactamase-*Klebsiella pneumoniae*
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
FIDSSA	Federation of Infectious Disease Society of South Africa
FS	Free State Province
FTP	File Transfer Protocol
GARP	Global Antibiotic Resistance Partnership

GASP	Gonococcal Antimicrobial Surveillance Programme
GERMS	Group for Enteric, Respiratory and Meningeal diseases Surveillance
GERMS-SA	Group for Enteric, Respiratory and Meningeal diseases Surveillance-South Africa
GP	Gauteng Province
GSH	Groote Schuur Hospital
GS	Gram Stain
HI	Haemophilus influenza
HJ	Helen Joseph Hospital
HMIS	Health management information system
IPD	Invasive Pneumococal Diseases
IT	Information Technology
JB	Johannesburg
KP	*Klebsiella pneumoniae*
KPC	*Klebsiella pneumoniae* carbapenemase
KZN	KwaZulu-Natal
LARS	Laboratory-based antimicrobial resistant surveillance
LIS	Laboratory Information System
LIMS	Laboratory Information Management System
LP	Limpopo
MDR	Multidrug-resistant
MEF	Middle Ear Fluid
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NASF	National Antimicrobial Surveillance Forum
NARMS	National Antimicrobial Resistance Monitoring System
NCCLS	National Committee for Clinical Laboratory Standards
NHLS	National Health Laboratory Services
NICD	National Institute for Communicable Diseases
NIOH	National Institute for Occupational Health
NNISS	National Nosocomial Infections Surveillance System
ODBC	Open Data Base Connectivity
PA	*Pseudomonas aeruginosa*
PBP 2a	Penicillin Binding Protein 2a

QA	Quality Assurance
QC	Quality Control
PHASA	Public Health Association of Southern Africa
RR	Risk ratio
RSA	Republic of South Africa
SA	*Staphylococcus aureus*
SAASP	South African Antibiotic Stewardship Programme
SASCM	South African Society of Clinical Microbiology
SBPAH	Steve Biko Pretoria Academic Hospital
S/I/R	Susceptible/intermediate resistance and resistance
SOPs	Standard Operating Procedures
SP	*Streptococcus pneumoniae*
SQL	Structured Query Language
TH	Tygerberg Hospital
TSN	The Surveillance Network –USA
UK	United Kingdom
USA	United States of America
UH	Universitas Hospital
VRSA	Vancomycin resistant *Staphylococcus aureus*
WC	Western Cape Province
WHO	World Health Organization

Preface

In November 2009, my supervisor, Professor Jill Murray, understanding my interest and passion in the field of infectious diseases, introduced me to the growing need to understand antimicrobial resistance surveillance among nosocomial bacteria in South Africa, and trying to answer the question 'whether laboratory information system can be utilised to monitor trends and patterns of resistance among nosocomial bacteria?'

This PhD thesis is submitted in fulfilment of the requirements for a Doctor of Philosophy Degree in the subject area of infectious Diseases Epidemiology. The thesis contains a compilation of original work done with the guidance of my supervisors: Professors' Jill Murray, Olga Perovic and Hendrik Koornhof. This research was conducted in line within the broader framework of the Antimicrobial Resistance Research and Surveillance for nosocomial bacteria which is run by Professor Olga Perovic.

The thesis presents comprehensive results of a research project that was undertaken in the Division of Epidemiology and Biostatistics, School of Public Health of the University of the Witwatersrand and the Antimicrobial Resistance Surveillance and Research unit of the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Services (NHLS). Appropriate acknowledgement of both financial and academic support that was provided during the course of the research has been highlighted. Where published work from other sources has been cited, appropriate referencing has been done.

Writing this thesis has not been easy due to its complex nature. I had to deal with a substantial scope of knowledge from multiple disciplines, in an attempt to provide a more comprehensive perspective on antimicrobial resistance surveillance among nosocomial bacteria in tertiary public hospitals in South Africa. These disciplines include microbiology, pharmacology, internal medicine, infectious diseases,

health informatics, epidemiology, public health, biostatistics, and data management. Despite such upheaval, in the process of putting together this thesis, I have realised that I have actually gained a wealth of knowledge in the field of antimicrobial resistance surveillance.

Chapter 1 Introduction

This chapter consists of an introduction, which details with the background, the statement of the problem and the justification of the study. This provides the extent of antimicrobial resistance and the reason for it being a public health problem. The chapter also gives background information on the value of surveillance for antimicrobial resistance among nosocomial pathogens, using the laboratory information system and the need to focus on nosocomial pathogens. Lastly, the aim and specific objectives of the study are highlighted, setting the road map of what has been done.

1.1 Background

Antimicrobial resistance (AMR) is undoubtedly emerging as a medical and public health challenge in most health care settings. (1, 2) Antibiotics have for years been effectively used for treating infectious diseases and saved millions of lives. (3) However, these gains have been reversed due to development of AMR, (4) and mortality due to resistant bacteria is considerably high thus adding to the increasing infectious disease burden. (5) Antimicrobial susceptibility pattern has changed over time and has to a great extent been propagated by inappropriate use of antimicrobial agents that has resulted in emerging resistance. (6)

Since 1941, when penicillin was introduced in the management of bacterial infections, AMR has progressively increased. (7, 8) The emergence of bacterial pathogens resistant to commonly used antibiotics is causing increasing concern because of its association with high levels of morbidity and mortality. (9, 10) High prevalence of resistance to antimicrobial agents impacts negatively on the patients and increases the burden on health care

expenditures, because of the need for additional diagnostic testing and longer duration of hospital stay. (11, 12)

The magnitude of drug resistance has raised the need for continued surveillance of antimicrobial susceptibility and to systematically monitor patterns and trends of antimicrobial resistance over time. Enhanced information retrieval and better understanding of the magnitude of the problem would facilitate timely implementation of appropriate interventions, including review of antimicrobial prescriptions policy and treatment guidelines that would reinforce prudent antimicrobial use. (13-15) In the face of down scaling of the development of new antimicrobial drugs by the pharmaceutical industry, the ultimate long-term goal of patient management, would be to cure patients and preserve the effectiveness of currently available antimicrobials so that they would remain functional for many years to come. (16)

There are various surveillance networks that have been established over the years, focusing on various pathogens that serve to provide reliable sources of antimicrobial susceptibility data. Such data have been used to determine resistance patterns and monitor emerging antimicrobial resistance both nationally and internationally. (17, 18) The national and international surveillance networks have focused on monitoring antimicrobial resistance patterns and trends over time of various bacterial pathogens that cause serious diseases in humans. However, at present there is paucity of data in most developing countries regarding the burden of antimicrobial resistance, even among nosocomial pathogens which reflect the situation in hospitals from where most resistance problems have emerged.

An effective electronic surveillance and monitoring system based on a laboratory information system (LIS) that aims to collect isolate-specific, good quality antimicrobial susceptibility

test results from clinical laboratories will substantially contribute to early detection of emerging antimicrobial resistance problems. To ascertain effective implementation of a suitable electronic surveillance system, it was imperative to carry out an evaluation of the usefulness and validity of LIS-generated data retrieved from the Corporate Data Warehouse (CDW) contracted by the National Health Laboratory Services (NHLS). To perform such an evaluation, the effectiveness of the existing LIS to determine the proportion of antimicrobial susceptibility of the clinical isolates obtained from hospitalized patients was interrogated. Relevant electronic data collected retrospectively over a 4.5-year period was used to study the epidemiology of antimicrobial resistance of selected bacterial pathogens that were known to be associated with increased proportion of multi-drug resistance in the hospitals.

The relevant bacterial pathogens chosen were S*taphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa*. The choice of these pathogens was in keeping with their association with multiple drug resistance and in-hospital acquisition. Furthermore they represent different spectra of drug susceptibility and therefore are exposed to different antimicrobial agents over time and, despite considerable overlap, acquire resistance to different antimicrobial agents. *S. aureus* was included because it represents Gram-positive bacteria and has a high mutation rate in genes encoding resistance to antimicrobial agents. It is also a very common cause of wound infection which not infrequently leads to blood stream invasion in hospitalized patients. It is a common commensal of humans and primarily lives in the moist epithelial layer of the anterior nasal area with a carriage rate of about 20% in the population. In its carriage capacity over time, as well as during invasive disease, *S. aureus* is frequently exposed to the selection pressure of antimicrobial usage. Carriage is an important risk factor for invasive infection, and significantly higher rates of *S.aureus* infection occur among hospitalized patients who have been catheterised as well as patients treated surgically. In addition methicillin resistant *S.aureus* is intrinsically resistant to methicillin and all β-

lactams, including the isoxazolyl penicillins as well as broad spectrum β-lactams and carbapemens. (19)

K. pneumoniae isolates were chosen as they are major emerging pathogens in nosocomial infections caused by Gram-negative bacteria belonging to the *Enterobacteriaceae* family. Unlike the *Escherichia coli* species which has a heterogeneous spectrum of pathogenicity (entero-toxinogenic, entero-invasive, entero-pathogenic, urogenic) and was another strong candidate for inclusion in the present study, *K. pneumoniae* has a narrower spectrum and is, in addition to wound infections and bacteraemia, associated with respiratory infections (pneumonia) and less commonly with urinary tract infections. High prevalence of extended spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* in hospital settings, poses an immense challenge in the clinical management of such infections, as treatment options are few due to the wide spectrum of antimicrobial resistance encountered in this organism. This makes *K. pneumoniae* an important bacterial species for inclusion in surveillance programs of hospital-acquired infections. (20)

P. aeruginosa is an opportunistic pathogen causing serious infections in hospitalized patients, mainly among immunocompromised patients with neutropenic patients as a special risk group. It is ubiquitous and its natural habitat is environmental niches where water or moisture is present e.g. shower tops, water taps and drains, flower vases etc. Treating *P.aeruginosa* is challenging due to inherent resistance to many antibiotics. It commonly produces ESBL enzymes, posing treatment difficulties. Furthermore, organisms such as *K.pneumoniae* and *P.aeruginosa* exhibit co-resistance to many other classes of antibiotics, resulting in the limitation of therapeutic options. (21)

Blood culture as the source of the three organisms, was chosen as isolates from this source (blood) signify bacteraemic episodes and are usually present as a single bacterial species in pure culture and are likely to be clinically relevant, as opposed to isolates from other body sites where the organisms may be merely present in a colonizing capacity, as opposed to causing invasive disease.

The outcome from this research: 1) provides a guide for the enhancement of a LIS for antimicrobial resistance surveillance in South Africa, 2) demonstrates the effectiveness of a LIS in capturing information related to antimicrobial susceptibility testing in microbiology laboratories, and 3) provides a platform for the reinforcement of an electronic based surveillance model appropriate for South Africa.

1.2 Statement of the problem

The rapid emergence and spread of multi-drug resistant community and nosocomial acquired pathogens is of great concern in both developed and developing countries. (22, 23) Management of infectious diseases in the 21st century is faced with major challenges associated with the use of antimicrobial agents. Recent studies have shown an increase in Gram-negative and Gram-positive organisms amongst nosocomial pathogens that are multi-drug resistant, and pose a great challenge for treatment and clinical management. (24, 25) While there are attempts by the pharmaceutical industry to manufacture new antibacterial agents which are expensive and beyond reach of most patients in developing countries, they invariably select for resistance, with a tendency to extend their selective pressure to involve resistance among similar drugs classes, diminishing their usefulness over time. The need for an effective surveillance system to elucidate the patterns and extent of drug resistance in

South Africa appears to be an appropriate intervention. Such a system would help to identify patterns and trends of AMR among nosocomial pathogens. (26, 27) The current LIS has not been utilized optimally and has shown significant deficiencies with regard to consistent and standardized data entry and reporting methodology, for it to be able to function efficiently.

1.3 Rationale of the study

Reliable information on the patterns of antimicrobial susceptibility of selected nosocomial pathogens in public sector hospitals in South Africa would be essential. Such information would aid in the assessment of the impact of antimicrobial resistance in the hospitals concerned and by extension potentially at the national level. In addition, information on AMR could be used in formulating beneficial prevention strategies on different health system levels in the country. The antimicrobial susceptibility surveillance data would provide a useful platform for planning targeted public health interventions to control spread of antimicrobial resistant pathogens in public sector institutions and guide future preventive as well as treatment recommendations.

To ensure this was achieved, the current LIS was evaluated checking for consistent, standardized data entry and reporting, as well as quality control methodology. This process helped in identifying the strengths and weaknesses of the system and gave direction on the appropriate ways of improving and making the system more effective. This study was done in line with the broad vision of laboratory-based antimicrobial resistant surveillance (LARS), whose focus had been to establish a functional integrated antimicrobial resistance surveillance system for commonly identifiable nosocomial pathogens in South Africa. Even though the focus of the study was on South African surveillance system for nosocomial

infections, other African countries would be in a position to implement a similar system for their antimicrobial surveillance. This study explored in greater depth the potential landscape of resistance surveillance in low income country settings and determined the feasibility of enhanced surveillance.

1.4 Literature Review

1.4.1 Basic Microbiology

1.4.1.1 Staphylococcus

The genus name *Staphylococcus* was derived from a Greek term *staphylos* which means a 'bunch of grapes', *coccus* means grain or berry (Figure 1.1). 'Staphylococcus' therefore implies that the cells of these organisms grow in clusters resembling clusters of grapes. However, in clinical specimens, the organisms may also appear as single cells, pairs, or in short chains. The genus contains over 30 different species and only three of these are of clinical significance: *S.aureus* (causes a wide range of major and minor infections in humans and its enzyme coagulase causes clotting of blood plasma), *S.epidermidis* (usually a skin commensal bacterium, causes opportunistic infection associated with prostheses or foreign body) and *S. saprophyticus* (causes urinary tract infection in healthy adult women). (28, 29)

The staphylococci organisms are 0.5-1.5 μm in diameter, non motile, facultative anaerobic (i.e. grow in both aerobic and anaerobic environments), and are able to grow in media containing a high concentration of salt i.e. 10% sodium chloride as well as in temperatures ranging from 10-40°C. Staphylococci are present on the skin and mucous membranes such as nasopharynx of humans and because shedding of this organism is common, it is responsible for the occurrence of nosocomial transmission among hospitalised individuals. (28)
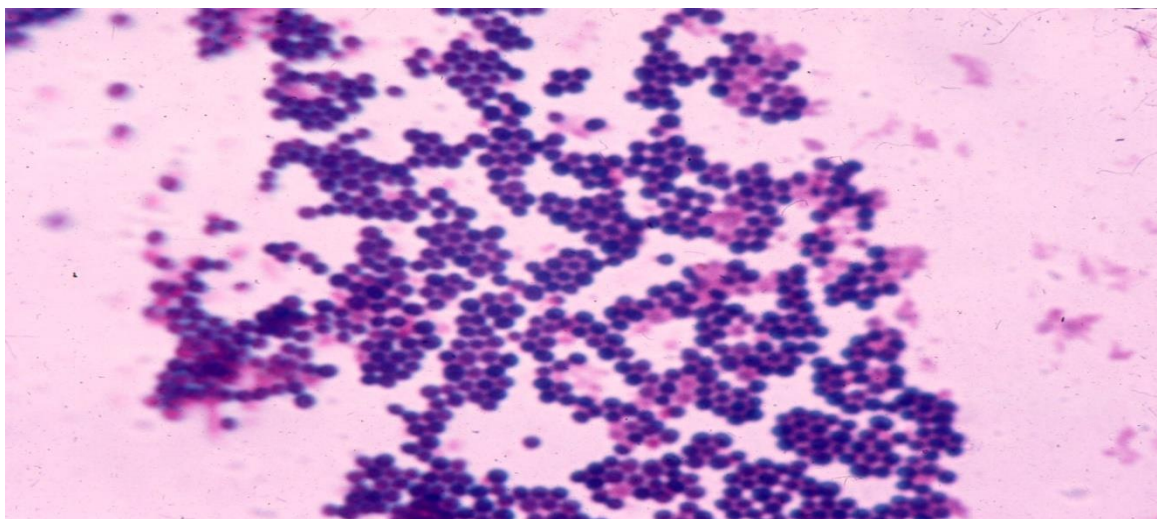
Figure 1.1 *Staphylococcus aureus* **resembling grain or berry-like in clusters like bunch of grapes** (Adapted from www.microbiologyinpictures.com/staphylococcusaureus.html).

S.aureus is a Gram positive *coccus*, about 1 μm in diameter. The organisms are non-spore forming, non-motile, and usually non-capsulate. The organism is non fastidious, capable of aerobic and anaerobic respiration. *S. aureus* causes the following clinical conditions: bacteraemia, osteomylitis, skin and soft tissues infection, pneumonia, toxic shock syndrome, surgical wound infection, and toxic epidermal necrolysis among others.

The treatments of choice for infection caused by *S.aureus* are penicillinase stable penicillins, since over 80% of hospital isolates are beta-lactames producers. (29, 30) The other challenge for treatment is methicillin resistance which has shown to be >30% in South African hospitals. (31) For these, vancomycin is indicated, but unfortunately emergence of vancomycin resistant *S. aureus* has been observed. (Chapter 6, table 6.2) The high rates of methicillin resistant isolates in many hospitals, is a major public health issue due to clinical implications of managing MRSA in the face of high resistance of first line treatment. This calls for active enhancement of surveillance of antimicrobial resistance among hospital isolates of *S. aureus* bacteria.

1.4.1.2 Klebsiella

Organisms belonging to the genus *Klebsiella* are capsulated, Gram negative rods, non-motile, approximately 1-2 μm in length (Figure 1.2). The capsule gives the mucoid appearance of isolated colonies and enhances virulence of organisms in vivo. *Klebsiella* genus belongs to the *Enterobacteriaceae* family, and the organisms are aerobic or 'facultatively anaerobic', ferment glucose and produce catalase but not oxidase. Hence the species and genera of the family of *Entrobacteriaceae* can be distinguished from each other by using biochemical tests in the clinical microbiology laboratory. The most commonly isolated members of this genus are *K. pneumoniae* and *K. oxytoca,* the latter being occasionally encountered in clinical specimens. (29, 30). The organisms grow at temperatures between 12°C to 43°C, and are found in the normal flora of the mouth, skin, and intestines.
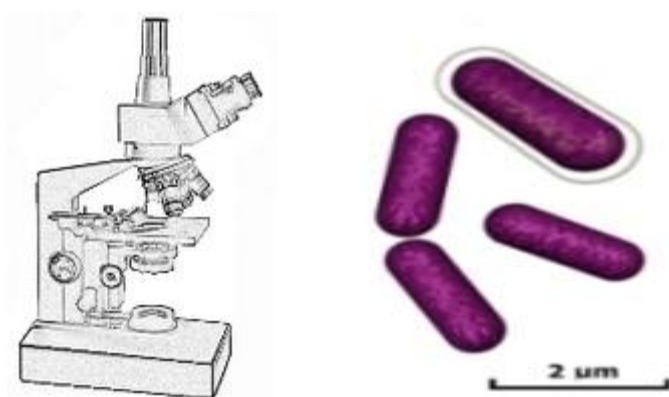
Figure 1.2 Microscopic appearance of encapsulated non-motile rod-shaped *Klebsiella pneumoniae* bacterium (**Adapted from www.microbiologyinpictures.com/klebsiellapneumoniae.html**)

*Klebsiella pneumonia*e is clinically the most important member of the *Klebsiella* genus of the *Enterobacteriaceae*. It is common cause of infections among immunocompromised hospitalised individuals. The organism causes among others the following illnesses: severe bronchopneumonia, bacteraemia and meningitis, and milder wound and urinary tract infections etc. Mortality associated with illness such as bacteraemia is high. (29) However, the main relevance of this micro-organism in humans is that it commonly causes surgical wound infections, urinary tract infection and bacteraemia among hospitalised individuals. The challenge in clinical management of these micro-organisms is related to plasmid

mediated multiple antibiotic resistances, limiting the choice of effective antimicrobial agents. (30) Regular surveillance to determine patterns of antimicrobial resistance would assist to guide empirical treatment and improve treatment outcomes.

1.4.1.3 Pseudomonas aeruginosa

This micro-organism is non-spore forming, motile by means of polar flagella, non-capsulated, straight or slightly curved Gram-negative rods typically arranged in pairs (Figure 1.3). The size of the micro-organisms measures 0.5 to 0.8 µm by 1.5 to 3.0 µm. The micro-organisms are saprophytic and found mostly in soil, water, and other moist environments. *P. aeruginosa* is an opportunistic pathogen and patients usually become infected through contact and spread if exposed to environmental sites colonised by these bacteria. The micro-organism typically produces a blue green pigment (pyocyanin) and a yellow-green pigment (pyoverdin). (28-30)

Figure 1.3 Appearance of *Pseudomonas aeruginosa* on Gram stain: rod-shaped cells arranged in pairs (Adapted from Todar's online textbook of bacteriology)

P. aeruginosa causes infections on the skin, burn wounds and nosocomial pneumonia in critically ill hospitalised individuals under intubation. In addition, *P. aeruginosa* can also cause bacteraemia, osteomylitis, endocarditis and urinary tract infection. *P. aeruginosa* is a common pathogen that causes nosomial infection among hospitalised individuals, and is a major lung pathogen among patients with cystic fibrosis. (30) Infections caused by

P.aeruginosa are clinically challenging to manage owing to intrinsic resistance to multiple antibacterial agents and the pathogen has a strong ability to acquire resistance from other antimicrobials during the course of treatment, particularly with prolonged broad spectrum antibiotics. (29, 32)

Owing to its inherent ability to develop resistance, effective monitoring patterns of resistance to antipseudomonal drugs would help to guide clinicians in their choice of antibiotics for empirical management of such infections, thereby enhancing infection control to minimise continued spread of resistant strains.

1.4.2 Mechanisms of action for antibacterial agents

Effectiveness of antibacterial agents can be sub-classified into four modes of action: i) interference with cell wall synthesis; ii) inhibition of protein synthesis; iii) interference with nucleic acid synthesis; iv) interference with the integrity of bacterial cell and outer membranes. (33) Antibacterial agents including beta-lactams comprising penicillins, carbapenems, cephalosporins, monobactams as well as glycopeptides which includes vancomycin and teicoplanin, exert their antibacterial effect through inhibition of bacterial cell wall synthesis. (33, 34). The beta-lactams inhibit bacterial cell wall synthesis through interfering with enzymes required for the synthesis of the peptidoglycan layer. Glycopeptides such as vancomycin and teicoplanin hinder bacterial cell wall synthesis through binding to the terminal D-alanine residues of the nascent peptiglycan chain creating an inability for the cross linking steps that are required for stable bacterial cell wall synthesis to take place. (34)

Other antibacterials such as aminoglycosides, macrolides, tetracyclines, chloramphenicol etc. produce their antibacterial effect through inhibiting bacterial protein synthesis. (33, 34) Bacterial ribosomes differ in structure from their counterparts in eukaryotic cells as such

antibacterial agents make use of these differences to selectively inhibit bacterial growth. Antibacterial agents such as tetracyclines and aminoglycosides bind to the 30S subunit of the ribosome, and agents such as macrolides and lincomycin as well as chloramphenicol binds to the 50S subunit.

Antibacterial agents in the fluoroquinolone group produce their antibacterial effect through disruption of DNA synthesis by DNA gyrase and topoisomerase IV enzymes, causing lethal double-strand DNA break during DNA replication process. (35) Others such as sulphonamides and trimethoprim interfere with folic acid synthesis leading to inhibition of DNA synthesis. The trimethoprim and sulfamethaxazole combination inhibits two steps in the enzymatic pathway for folic acid synthesis by bacterial cells. This dual combination potentiates the antibacterial activity of one another through synergistically acting against an array of pathogenic bacteria. (36) Lastly, disruption of bacterial membrane structure is another recognised mechanism of action and forms the basis for the action of polymyxins. It is hypothesised that polymyxins exert their inhibitory effects through increasing bacterial membrane permeability leading to leakage of bacterial contents and eventual death of bacterium. (37)

1.4.3 Mechanisms of Antibacterial Resistance

There are a variety of mechanisms by which bacterial can manifest resistance to antibacterial therapy. The following are some of the ways bacteria can manifest resistance: i) The bacteria might acquire genes encoding enzymes, such as beta-lactamases, which are enzymes that neutralize the activity of beta-lactam molecules before they exert their effects against susceptible bacteria; ii) The bacteria might acquire efflux pumps that remove the antibacterial agent from the bacterial cell before it reaches and exerts its effect at its target site; iii) The bacteria might acquire genes for a metabolic pathway that eventually produces altered

bacterial cell walls that no longer contain the binding site of the antibacterial agent or the bacteria might acquire mutations affecting ribosomes which might limit access of antibacterial agents to their intracellular target sites. (38) In this situation, bacteria that are normally susceptible might acquire resistance to antibacterial agents through mutation and selection or through acquiring from other bacteria the genetic information that encodes resistance. This might occur through genetic transfer mechanisms that includes transformation, transduction or conjugation. (38)

Bacteria susceptible to antibacterial agents can acquire resistance via new mutations. (34) These bacterial cell mutations might cause resistance through: i) altering target protein to which antibacterial agents binds through modifying the nature of the binding sites (such as a change in penicillin-binding protein *2b* in pneumococci that results in pneumococcal resistance to penicillin by homologous recombination of DNA from oral streptococci and not by mutation); ii) up regulating the production of enzymes that inactivate the antimicrobial agents (for example erythromycin ribosomal methylase in staphylococci and more importantly, beta-lactamases and cephalosporinases that hydrolise beta-lactam agents acting on bacterial cell wall synthesis); iii) down regulating or altering an outer membrane protein channel that antibacterial agents require to gain entry into the cell (for example, OmpF porin in *E. coli* for fluoroquinolone resistance); iv) up regulating efflux pumps that drive out antibacterial compounds from the bacterial cell (i.e. efflux of fluoroquinonolones in *S. aureus* and *P. aeruginosa*). (34)

In all the scenarios presented above, bacterial strains carrying resistance-conferring mutations are selected through antibacterial use that selectively kill susceptible strains and allows the

new resistant strains to survive and multiply. This phenomenon of acquired resistance that develops as a result of chromosomal mutation and selection is called 'vertical evolution'.

In addition, bacterial pathogens also develop resistance through acquisition of new genetic material from other resistant organisms, a process called 'horizontal evolution' or horizontal gene transfer of resistance. In this case also, as is the case with vertical evolution, mechanisms of genetic transfer include: conjugation, transduction and transformation. For each of these mechanisms, transposons, plasmids, or integrons might facilitate transfer of resistance genes between bacterial strains or species, leading to acquired resistance. (34) Mutation and selection, together with the mechanisms of genetic exchange, enable many bacterial species to adapt quickly to the introduction of antibacterial agents into their environment, developing antibacterial resistance in the process.

1.4.4 Overview of studies quantifying impact of nosocomial infections

Nosocomial infections, also called "hospital-acquired infections", are infections acquired after more than 48 hours of patient admission in the hospital and such infections should not be present or incubating at the time of admission. (39) Nosocomial infections are an important cause of morbidity and mortality and have become a major focus of infection prevention across the globe in recent times. (40) Such infections are increasingly becoming important public health problems due to increasing economic impact in populations since such infections are associated with development of antimicrobial resistance to several drug classes. (41)

Occurrence of nosocomial infection in hospitalised patients come as a result of interrelationship of several factors including, but not limited to: compromised immunity among patients; invasive surgical/medical procedures; poorly ventilated and overcrowded

hospital wards. In addition, transmission of infection in hospitals can also be facilitated by poor infection control practices. Nosocomial infections are one of the leading causes of death (42-48) and are associated with high economic costs due to long hospital stay. (49-51)

Previous studies documented prolonged duration of hospitalisation due to acquisition of nosocomial infections. (48) Prolonged hospital stay increases direct hospital costs and indirect costs due to loss of work resulting from unproductivity of the patient. Prolonged admission is directly linked to increase in drug use, requests for additional laboratory and other diagnostic tests, as well as nursing-related and other costs which all lead to increased financial burden.

The selective pressure of intense antibiotic use promotes emergence of antibiotic resistance, (49-51) underpinning the importance of antimicrobial stewardship that would enhance prudent use of antibiotics. (52, 53) This underscored the need for an extensive investigation into current trends of antimicrobial susceptibility among common nosocomial pathogens. Therefore performing an analysis of laboratory-based surveillance data aggregated from various participating clinics/hospitals through an electronic surveillance system is an ideal approach to unravel antimicrobial resistance patterns and trends over time in health care settings. (41) Such information would guide establishment of appropriate and effective AMR control measures.

1.4.5 Laboratory Information System

1.4.5.1 Definition

A laboratory information system (LIS) is a computer-based application software product that is used in the laboratory to manage analyses and standard samples, tests results, laboratory staff and analytic equipment, as well as for the purpose of generating commercial reports and other functions. (54) In this thesis LIMS (Laboratory information management system) and LIS will be used interchangeably as the two terms have subtle differences when used in certain contexts.

The roots of LIMS were in laboratory automation. (55) The standardisation and updating of the medical laboratory testing procedures are the fundamental guarantees to the quality of test results. Therefore, LIS is formed from the connection of a variety of analytical instruments based on the network information system software, and its quality control function should meet the requirements of clinical laboratory quality assurance. LIS is a powerful software system and include a series of functions such as patient information database, data reception, quality control data management, data analyses and laboratory management. LIS has played an important role in laboratory management and improving the efficiency of laboratory routine work and ensure the reliability of laboratory data. (56)

1.4.5.2 Objectives of LIMS

The objectives for the establishment of LIMS were: i) to increase productivity, ii) to enhance laboratory compliance with Good Laboratory Practice guidelines, iii) to eliminate calculation and transcription errors, iv) to achieve automated generation of reports and integration with word processing, v) to speed up the interpretation of data, vi) to bar code labels for samples and rapid data entry, vii) to verify data entry, viii) to achieve a flexible and expandable

system, xi) for the system to incorporate sample tracking and scheduling, x) to accommodate on-line access to historical analytical data. (57)

1.4.5.3 Historical basis

The development of LIMS has spanned over 30 years. The first of such systems were chromatography data systems, developed and introduced by major manufacturers of analytic equipment's such as Hewlett Packard, Perkin Elmer, and Beckmann Instruments. (54, 55) Introduction of computer technologies in late 1970s and early 1980s enhanced the process of laboratory information management. The technology of LIMS developed from in-house software products aimed to meet certain needs. There are now over 100 firms across the globe that produces such software products and utilise a number of specification documents such as standards for LIMS that regulate their activity. (58)

1.4.5.4 Main Functions

The main functions of LIS are operated interactively on a mini computer which enables the laboratory complete control over daily processing. Collection of results from automated analysers is accomplished via a micro-processor network. Links are provided to a central main frame computer for immediate patient on-line identification and historical data processing. LIS is designed to manage all the operations involved in laboratory activities; in our situation we can give an example of the central computer as the Corporate Data Warehouse (CDW). The system has 14 main components, as outlined below. (59)

1. Registration of test requests

2. Production of specimen collection sheet and identification labels

3. Confirmation of specimen collection

4. Production of aliquot labels

5. Work load inquiry

6. Production of worksheet

7. Manual entry of test results entry

8. Automated entry of test results

9. Results inquiry

10. Preliminary report

11. Final report

12. Daily activities reports

13. Statistical reports

14. Billing

The main functions of LIS are as listed above. Functions 1, 3, 7 and 8 are the main input processes. They are used to select tests to be done according to the request form, to notify when the necessary specimen becomes available, and register the corresponding test results after analysis.

The collection sheet includes specimen identification labels to be put onto specimen tubes. The collection sheet, identification labels, aliquot labels, and worksheet are designed to diminish clerical work in the laboratory as well as in laboratory-related activities. All the above functions are used intensively during daily routine along with maintenance functions like test request collection. Statistical reports and billing are produced on a periodical basis.

The mini computer software is designed in a modular manner. Most features of the system can, therefore, be adapted to the needs of the user by setting the proper information in the corresponding module and this can be accomplished without any change to the program code.

Furthermore, the user can make the system evolve with changing needs, altering the parameters value. (59)

1.4.5.5 System security

To safeguard the LIS, the system security is provided along the following lines: data entry validation, system access control and memory protection.

1.4.5.6 Main advantages

The main advantages of LIS that could be realised by the laboratories include: i) reduced clerical workload; ii) improved evaluation of workload, iii) faster communication, iv) improvement of information given to clinicians: adapted reference values, interpretation, comments, v) improved retrieval operations, vi) faster billing; data storage, ease of data manipulation and data integrity. All this together produces increased productivity of the laboratories.(57, 59) Details of LIMS work flow are presented in figure 1.4 below.
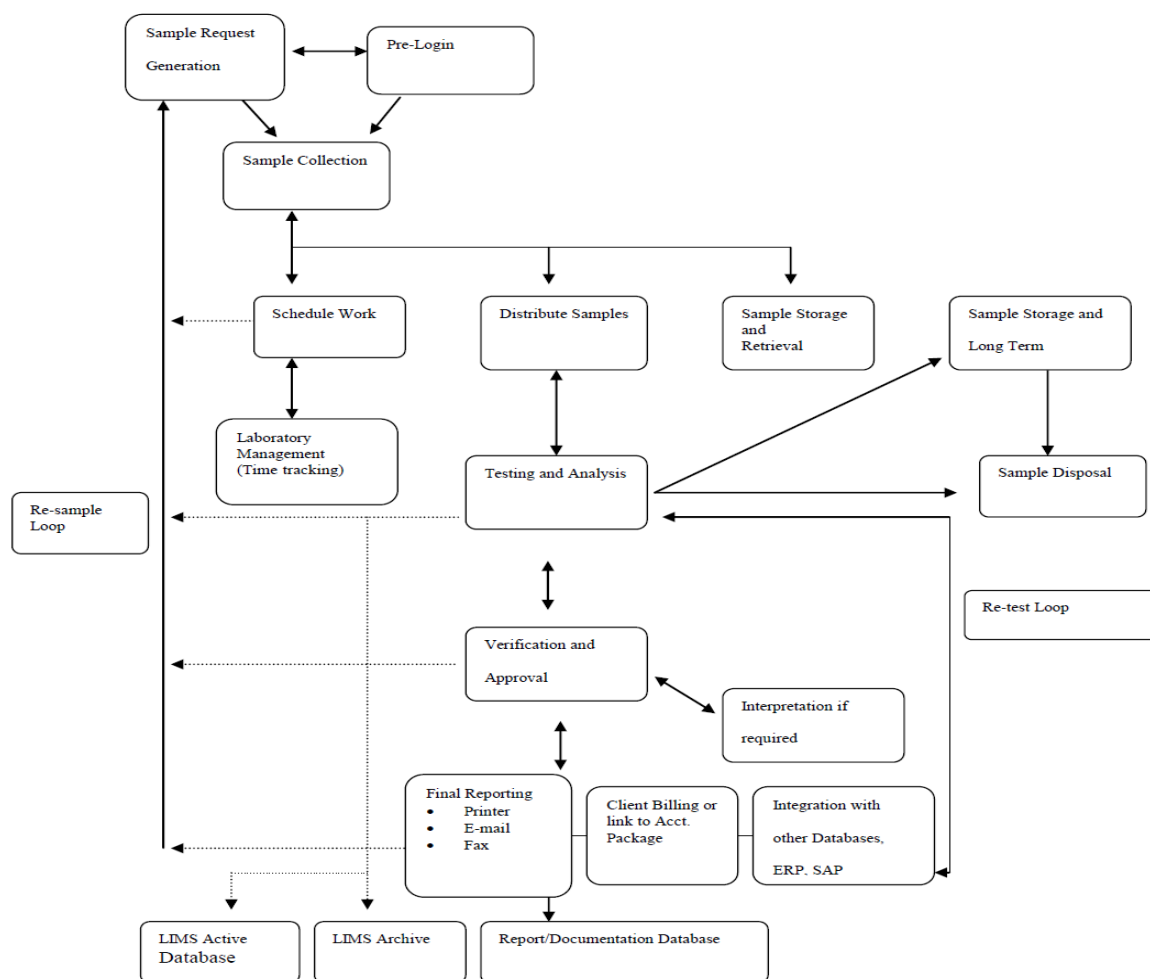
Figure 1.4 Schema representing generic LIMS work flow.

Figure 1.4 reproduced with permission from Christine Paszko, Ph.D., Vice President of Sales & Marketing,

Accelerated Technology Laboratories, Inc., West End, NC 27376 (Email address: CPaszko@atlab.com)

1.4.5.7 Public Health Function of LIS

In industrialised countries, LIS is a component of the clinical and public health laboratory
infrastructure. The system helps to monitor disease profiles, including chronic and infectious
diseases, but can also be used to monitor development of antimicrobial resistance through
standardised and integrated laboratory-based surveillance. A properly-designed health
management information system (HMIS) comprising of reliable, accurate and timely
available data is widely viewed as the pillar of a good public health system.

Integrating the LIS into HMIS can effectively support several public health functions and programs such as epidemiology, surveillance and monitoring, assessment of outcomes, policy analysis, research, program planning and evaluation among others. (41, 60, 61) A LIS is therefore crucial as a system capable of maintaining an integrated data flow between various health facilities e.g. focusing on diseases of outbreak potential, but can also facilitate prompt delivery of results to clinical care providers at all levels of care. Through a reliable computerised system for laboratory data entry, access and retrieval of data would be ideal in addressing important issues, such as sample tracking, automated delivery of patient reports as well as generation of aggregate reports among other functions. (62)

In this regard, data from an integrated network of NHLS clinical laboratories using an electronic surveillance system that collects microbiological data from all clinical microbiology laboratories across the Republic of South Africa could be a reliable source of data for analysis. This would include comprehensive information that could provide valuable insights into patterns, trends over-time and prevalence of important micro-organisms associated with antimicrobial resistance problems. Good examples would be *S. aureus, K. pneumoniae* and *P. aeruginosa* micro-organisms, which could serve as indictator bacteria for antimicrobial resistance surveillance in hospital settings. Such surveillance would allow investigating the extended spectrum beta-lactamase (ESBLs), methicillin-resistant *S. aureus* (MRSA) and other multidrug resistance patterns and trends according to patient location, geographic distribution and specimen source. A well established LIS would therefore, in the long run, improve health system functions and delivery of quality care through strengthening larger public health systems and ensure proper utilisation of data for better clinical as well as public health outcomes. (41, 62) Figure 1.5 below illustrates the critical steps for the implementation of an effective nationwide LIS.
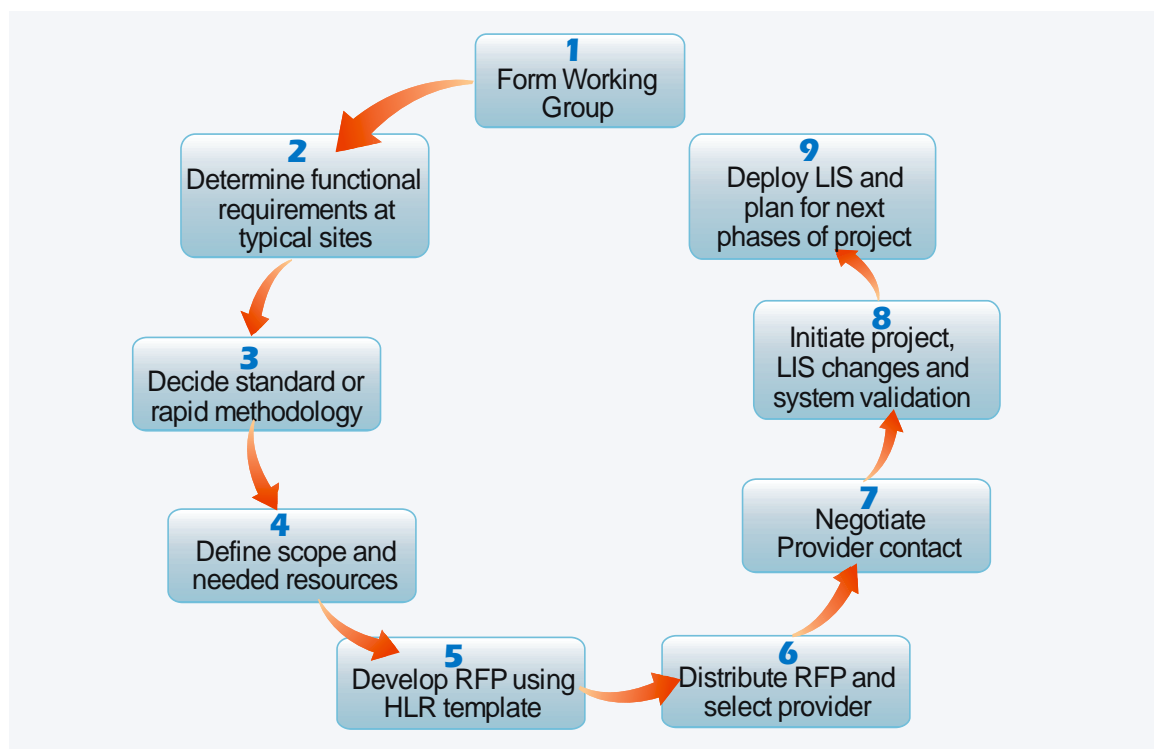
Figure 1.5 Critical steps to implement an effective LIS nation wide. HLR: High Level
Requirements; RFP: Request For Proposal

Adapted from: Guide book for Implementation of Laboratory Information Systems in Resource Poor
Settings. Association of Public Health Laboratories Publication, January 2006 (41)

1.4.6 Surveillance Systems for Antimicrobial Resistance

Public Health Surveillance is defined as the 'ongoing and systematic collection, analysis and
interpretation of outcome-specific data essential to the planning, implementation, and
evaluation of public health practice, closely integrated with timely dissemination of these
data to those who need to know; and the application of these data to the control and
prevention of human disease and injury'. (63) Even though few international antimicrobial
resistance surveillance initiatives seem to correspond to this definition (17), in the context of
this definition, major challenges exist including lack of appropriate denominator data. In
addition variation in blood culture taking practices when LIS is based on bacteraemic
episodes, are barriers to the establishment of an effective surveillance system and is viewed

to be a low-cost tool used to generate locally valuable information on antimicrobial resistance profiles. The ideal surveillance system therefore, can also function as a quality assurance tool, bridging standardized reporting and methodological issues in identification of resistant pathogens as well as improve the quality of antimicrobial susceptibility testing. (64)

1.4.6.1 Surveillance Initiatives for antimicrobial resistance in South Africa

For many years, South Africa has had no active and functional national electronic surveillance system for monitoring antimicrobial resistance in hospital acquired infections. The South African Antibiotic Stewardship Programme (SAASP) has been instrumental in reinforcing the prudent use of antimicrobials through the antimicrobial stewardship. Some issues on how to contain resistance have been previously handled by the South African Society of Clinical Microbiology, formerly National Antimicrobial Surveillance Forum (NASF). This grouping has been involved in passively collating antimicrobial data in public and private health care sectors. An established entity within NHLS situated at the National Institute for Communicable Diseases (NICD) called the Group for Enteric, Respiratory and Meningeal diseases Surveillance (GERMS-SA) operating in all of the nine provinces also focuses on surveillance of community-acquired pathogens and monitors resistance profiles. Another initiative was introduced in KwaZulu-Natal (KZN) for surveillance of *E. coli* in 2000/2001 (65) and also the Veterinary Surveillance of Antimicrobial Resistance in South Africa. (66)

As of 2010, the Antimicrobial Resistance Surveillance and Research (ARSR) within the Centre for Opportunistic, Tropical and Hospital Infections was established at NICD to run an active laboratory based surveillance system for monitoring resistance to nosocomial pathogens realizing the increasing burden of hospital acquired infections commonly

associated with high rates of resistance. These are the main initiatives undertaken towards continued monitoring of antimicrobial resistance in South Africa.

1.4.6.2 International surveillance networks for antimicrobial resistance

Increasing antimicrobial resistance poses a major threat to global public health. Therefore, collective action is required to secure an effective multinational antimicrobial surveillance system to monitor the antimicrobial resistance profile and ensure rational use of antimicrobials. (67) The global antimicrobial resistance challenge, equally requires a concerted multinational force. This is in view of the negative impact of globalization at spreading infectious diseases vis-à-vis spreading of antimicrobial resistance. (16, 68) No country acting independently can effectively contain antimicrobial resistance.

It was in this context that the Global Antibiotic Resistance Partnership (GARP) initiative was established so that countries could actively work together to generate surveillance data that could be analyzed to predict or show trends of antimicrobial resistance. (69) The SENTRY Antimicrobial Surveillance Program was instituted in 1997 to monitor trends in antimicrobial resistance patterns in nosocomial and community-acquired infections worldwide so as to define appropriate control measures for antimicrobial-resistant pathogens. (70) The Alexander Project, an international surveillance network examining trends and patterns of antimicrobial susceptibility for community-acquired respiratory tract infections, begun its work in 1992. (71-76) The European Antimicrobial Resistance Surveillance System (EARSS) (77) currently known as the European Antimicrobial Resistance Surveillance Network (EARS-Net) (78), founded in 1998, is a pan European antimicrobial surveillance network which has been focussing on major invasive pathogens of clinical and epidemiological

relevance and provides antimicrobial resistance data that are validated and comparable across the network. (79)

The World Health Organization (WHO) has over the years instituted several antimicrobial resistance surveillance networks to contain development and global spread of antimicrobial resistance. (15, 16, 60) These include a Collaborating Centre for Surveillance of Antimicrobials (CCSA), which was instituted for the purpose of monitoring trends of antimicrobial resistance among various bacterial pathogens; (60) such as the Gonococcal Antimicrobial Surveillance Programme (GASP), initiated to contain antimicrobial resistance encountered in gonococci infections and the WHONET software designed for management and analysis of antimicrobial resistance data. (80)

The initiatives detailed above are among the most well known initiatives that have been established to monitor and provide objective data on antimicrobial resistance trends and profiles, in order to use such data to develop strategies to contain the continued development and spread of antimicrobial resistance around the globe. Table 1.1 provides a detailed overview of major national and international surveillance networks established for purposes of antimicrobial resistance surveillance.

Table 1.1 Major Surveillance Networks for Antimicrobial Resistance

Name	Acronym	Pathogens involved	Web address	Extent of Surveillance
European Antimicrobial Resistance Surveillance System	EARSS	*S. pneumoniae, S aureus, Enterococcus, E. oli, K.pneumoniae, P.aeruginosa.*	http://earss.rivm.nl/	Pan-European
The Surveillance Network –USA	TSN	*S. aureus*	http://eurofinsmedinet.com	USA, Europe
Canadian Bacterial Surveillance Network	CBSN	*S. pneumoniae,* *H.influenzae*	http://microbiology.mtsinai.on.ca/research/cbsn	Canada
World Health Organization: • Centre for Surveillance of Antimicrobial Resistance • WHONET software • Surveillance of antimicrobial resistance • Gonococcal Antimicrobial Surveillance Programme	WHO GASP	Various Pathogens An information system for monitoring antimicrobial resistance *Neisseria gonorrheae* only	http://who.int/drugresistance http:/who.int/drugresistance/whonetsoftware/en/ http://who.int/drugresistance/surveillance/en/ http://who.int/hiv/strategic/surveillance/en/gasp1998.pdf	International
Antimicrobial Surveillance Program for Asia-Pacific region & South Africa	SENTRY	*S. pneumoniae, H. influenzae, M. catarrhalis*	http://health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-cdi-2003-cdi27suppl-htm-	International
Centres for Disease Control • National Antimicrobial Resistance Monitoring System • National Nosocomial Infections Surveillance System	 NARMS NNIS	 For enteric bacteria Only isolates associated with nosocomial infections	http://cdc.gov/narms/	USA
Alexander Project		For community acquired lower respiratory tract pathogens		International
Surveillance Initiatives for Antimicrobial Resistance in South Africa				
Group for Enteric Respiratory and Meningeal Pathogens	GERMS-South Africa	For community acquired and hospital pathogens	http://nicd.ac.za/units/germs/germs.htm	National/South Africa
South African Society of Clinical Microbiology	SASCM	Monitoring of antimicrobial resistance patterns in the public and private medical sector in South Africa	http://fidssa.co.za/A_NASF_Overview.asp	National/South Africa
Veterinary Surveillance of antimicrobial resistance in South Africa			http://www.ncbi.nlm.nih.gov/pubmed/15580774	National/South Africa
Global Antibiotic Resistance Partnership	GARP	Global antimicrobial resistance monitoring initiative	http://resistancestrategies.org/wpcontent/uploads/2010/04/GARP-SA-8-9-Feb-Agenda.pdf	International

1.4.7 Surveillance of selected pathogens of Clinical and Epidemiological Relevance

1.4.7.1 Methicillin-resistant S. aureus (MRSA)

Data are scarce on the antimicrobial susceptibility profiles of South African MRSA isolates in both public and private health care systems. The Marais et al, study which is the first report on antimicrobial susceptibility patterns in MRSA isolates in South Africa, showed antibiotic resistance of MRSA ranging between 55% and 78% to tetracycline trimethoprim/sulfamethoxazole, erythromycin, gentamicin and ciprofloxacin, respectively. All isolates in this study were susceptible to teicoplanin, linezolid, vancomycin and quinopristin/dalfopristin. (26) It was therefore imperative to establish MRSA prevalence in South Africa.

As a step in that direction, the Antimicrobial Resistance Surveillance and Research (ARSR) unit was initiated at the National Institute for Communicable Diseases with a goal to determine the proportion of *S. aureus* strains resistant to methicillin in blood isolates from tertiary public hospitals associated with academic institutions in South Africa. The ARSR performs minimum inhibitory concentration (MIC) determinations of vancomycin against *S. aureus* strains, using the micro-dilution, agar-dilution or Etest methods. These methods are also used to determine the susceptibility of isolates to rifampicin and linezolid which are the most common alternative treatment options for MRSA. The key element would be to report antimicrobial susceptibility test (AST) results on the primary isolate from the blood specimen obtained during a bacteraemic episode which on identification proves to be a coagulase-positive *S. aureus*. (77, 81)

For reliable results of MRSA susceptibility testing, protocol recommends the use of a cefoxitin disk diffusion test or to alternatively use the oxacillin agar screen plates or the

oxacillin disk diffusion test even though the latter is less reliable. It is recommended that participating laboratories in the antimicrobial resistance (AMR) surveillance program report susceptible, intermediate or resistant (S/I/R) isolates tested on the basis of Clinical and Laboratory Standards Institute (CLSI) guidelines including interpreting the MIC or zone inhibition diameter if at all possible. Invasive *S. aureus* isolates from blood should be reported per patient per quarter. There is no need to report a second isolate during a febrile episode, even if the susceptibility pattern is different from that of the first isolate. (77)

1.4.7.2 Klebsiella pneumoniae and extended spectrum beta-lactamases (ESBLs)

There is a paucity of data on antimicrobial susceptibility profiles of blood culture isolates of *Klebsiella pneumoniae* in the South African public health care system. Brink et al, were the first authors to report on antimicrobial susceptibility of blood culture isolates in private sector hospitals in South Africa and they showed that the overall proportion of resistance to *Escherichia coli* blood culture isolates to ampicillin and fluoroquinolones was 84% and 20% respectively, and a further 5% of ESBL production among all isolates of *E. coli* in private health care institutions. Even though *E. coli* is important, our primary focus would be on *K. pneumoniae*, a Gram-negative bacterium and the main producer of extended spectrum beta-lactamases (ESBLs). (27) Information on levels of resistance to conventional antibiotics is essential for public sector health care institutions in South Africa, as it would help guide appropriate empirical therapy for invasive bacterial infections.

Successful antimicrobial resistance surveillance (AMRS) requires reliable reporting of AST results for the primary isolates of *K. pneumoniae* from blood culture per each patient investigated. If the antimicrobial resistance surveillance were to succeed, regular reporting to monitor susceptibility patterns of such invasive organisms is vital. Ideally, the first invasive *K*

pneumoniae isolate from blood per patient per quarter should be reported, and differences in susceptibility patterns in blood culture isolates need to be noted and reported. (77)

1.4.7.3 Pseudomonas aeruginosa

There are scanty data available on antimicrobial susceptibility patterns of isolates of *Pseudomonas aeruginosa* in public health care institutions in South Africa. A study done by Perovic et al, at an academic hospital in Johannesburg, South Africa showed an association between *P. aeruginosa* bacteraemia and outbreaks caused by multiple-resistant genotypes. In this study, 57.1% of the patients had a nosocomially-acquired infection. (82) The prevalence and extent of antimicrobial resistance among *P.aeruginosa* bacteria in public sector health care institutions in South Africa still remains unknown, since the Perovic et al, study only looked at data from 1998-1999 and this is over a decade ago. The resistance profiles and incidence of disease might have changed with time and the current status might be different. For the AMRS program to be effective, it is required to report AST results for the primary isolates of *P. aeruginosa* from blood cultures per each patient investigated. (77)

1.5 Research Question

This study was set to answer the question "Can the NHLS DISA laboratory information system or equivalent be utilized for surveillance of antimicrobial resistance for nosocomial pathogens in public tertiary hospitals in South Africa?"

1.6 Aim of the study

The aim of this thesis is to assess utilization of the LIS at NHLS, as a tool for reporting antimicrobial resistance (AMR) and monitoring resistance patterns and trends over time, for clinical blood culture isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolated from patients admitted in tertiary public hospitals in South Africa.

1.6.1 Specific Objectives

The specific objectives are outlined below with a description of how they were studied:

1. Conduct a systematic review with the aim of finding out the prevalence of antimicrobial drug resistance among *S. aureus, K. pneumoniae*, and *P. aeruginosa* from published literature, and to understand whether or not such data were part of an ongoing surveillance system for nosocomial infections in South Africa.

2. Perform a critical analysis of the utility of LIS of the NHLS as a surveillance tool for antimicrobial resistance profiles in selected nosocomial pathogens (isolated during bacteraemic episodes from blood cultures of patients in tertiary public hospitals in South Africa, retrieved from the Central Data Warehouse (CDW) of the NHLS from Mid 2005 to December 2009):

i)   Describe the strengths and limitations of the use of routine laboratory data relating to blood culture isolates of the selected pathogens through assessing simplicity,

flexibility, data quality, acceptability, sensitivity, positive predictive value, representativeness, timeliness, completeness and stability.

ii)   Assess laboratory methods and how they would impact on antimicrobial resistance surveillance.

3. Evaluate the suitability of the NHLS LIS as a monitoring tool for recording antimicrobial resistance trends and patterns in tertiary public hospitals in South Africa. To achieve this, we carried out data analysis to:

i)   Describe the demographic profile of patients with clinical isolates of selected pathogens admitted in tertiary public hospitals in South Africa, from mid 2005 to December 2009.

ii)   Determine antimicrobial resistance patterns of selected pathogens at different tertiary public hospitals in South Africa, from mid 2005 to December 2009.

iii)   Detect emergence of resistance and monitor trends over time of antimicrobial susceptibility of selected pathogens in tertiary public hospitals in South Africa from mid 2005 to December 2009.

iv)   Describe the potential pitfalls of the LIS as a surveillance tool for monitoring antimicrobial resistance using CDW data from mid 2005 to December 2009.

4. Compare prospectively antimicrobial resistance data submitted from tertiary public hospitals to the ARSR Unit over a 12-month period, from July 2010-June 2011 (as the gold standard) with antimicrobial resistance data retrieved from the CDW for the 2005 to 2009 period. To achieve this objective we aimed to specifically:

i)   Determine the proportion and patterns of antimicrobial resistance of selected pathogens over a 12 months period in tertiary public hospitals in South Africa between 1st July 2010 and 31st June 2011.

ii)   Compare patterns of antimicrobial resistance data to various antimicrobials that are commonly used in hospitals from July 2010 to June 2011 period, with retrospective (2005 to 2009) period, so as to ascertain reliability of routine data sources.

5. Describe how the question of AMR surveillance is being dealt with in another African country such as Tanzania, in East Africa.

Chapter 2   Study Methods

In this chapter, a detailed outline of the general study methods is provided, which consists of an in-depth description of the laboratory based investigation, the study design, sampling, data acquisition from the CDW, data management, data analysis methods, ethical consideration for use of secondary data as well as general limitations of the study methods.

2.1 Laboratory based surveillance

Pathogens isolated from blood were tested for susceptibility to commonly used antimicrobial agents at NHLS laboratories. These were preferred as the sensitivity at identifyinbg pathogenic bacteria is higher than other specimens. Susceptibility testing was performed in tertiary hospitals using existing methods such as disk diffusion and/or in vitro minimum inhibitory concentration (MIC) determinations, with internationally accepted breakpoint concentrations denoting susceptibility, intermediate resistance and full resistance. Antimicrobial susceptibility of these pathogens was assessed using the following antimicrobials agents: broad spectrum penicillins, including amino-ureido - and isoxazolyl penicillins, carbapenems and ß-lactamase inhibitors plus ß-lactam antibiotics, 3rd generation cephalosporins, fluoroquinolones, aminoglycosides, glycopeptide, chloramphenicol and trimethoprim/sulphamethoxazole. (26, 82)

2.2 Study design and setting

This was a retrospective record review covering the period 2005 to 2009 with a prospective component conducted during period July 2010 – June 2011 that used data of clinical isolates of selected bacterial pathogens collected at seven NHLS sites situated at tertiary public

hospitals linked to academic institutions in three provinces in South Africa. The sites were: i) from Gauteng Province, Charlotte Maxeke Johannesburg Academic (CMJAH), Chris Hani Baragwanath (CHBH), Helen Joseph (HJH), Steve Biko Academic Hospital (SBAH) linked to Witwatersrand and Pretoria Universities; ii) from Free State Province, Universitas Hospital linked to the Free State University; iii) from Western Cape Province Hospitals, Groote Schuur Hospital (GSH), and Tygerberg Hospital (TH) linked to Cape Town and Stellenbosch Universities, respectively.

Two criteria were used in determining inclusion of laboratories into this study i) that they were linked to reputable academic institutions (Wits, UCT, Free State, SUN, UP) with good quality assurance practices of laboratory methods likely to yield reliable results as well as computerised laboratory systems from which data required for the present study could be readily accessed; ii) laboratories that were interfaced to the CDW in Johannesburg and are part of the NHLS but also cover a wider spectrum of patients infected with the three selected organisms exhibiting antimicrobial resistance. The map below (Figure 2.1) highlights population size of each province that formed part of our population from which the bacteraemia patients were drawn. The population size might have been a determinant of the number of isolates from each province, with more isolates coming from Gauteng, then Western Cape and lastly Free State.
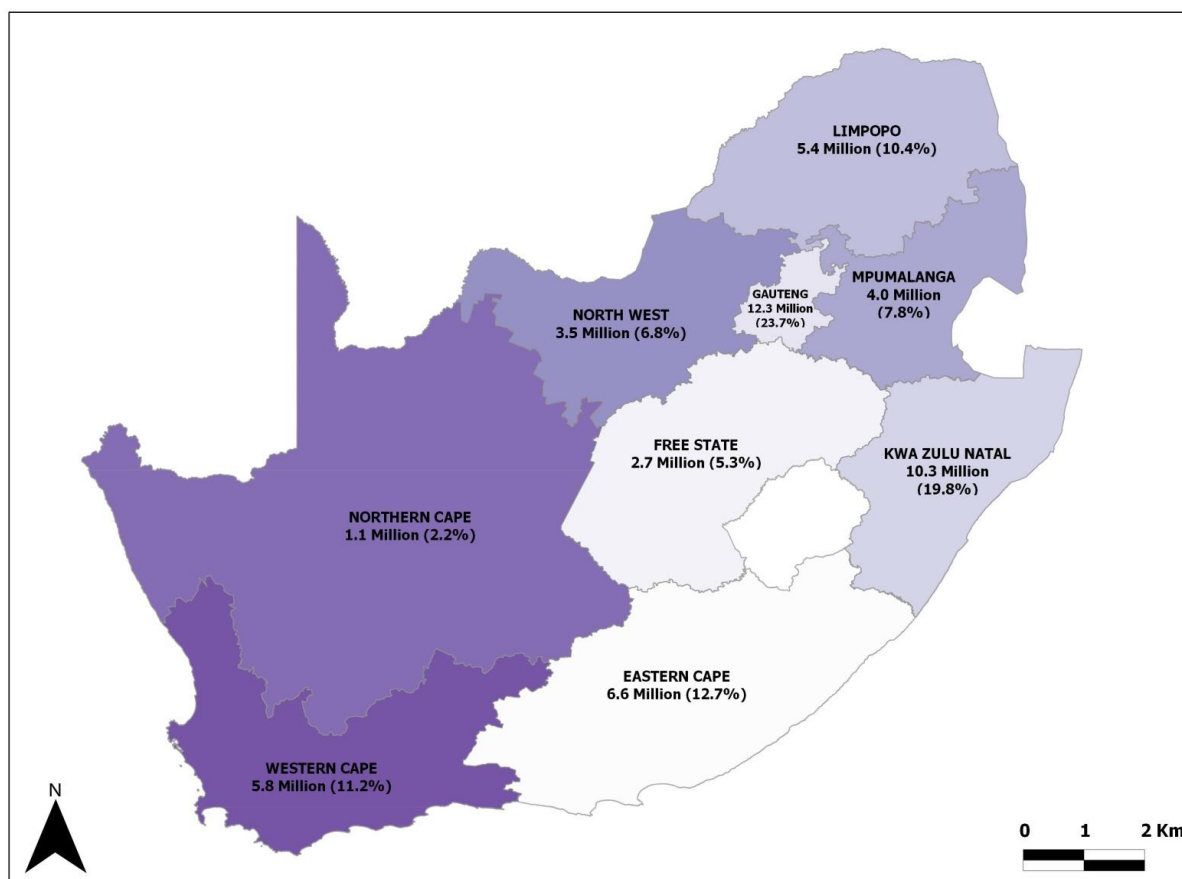
Fig: 2.1 Map of the Republic of South Africa showing population distribution in each Province

2.3 Sampling and sample size

The study used all records of blood culture isolates from the seven hospitals submitted to and reported by the NHLS laboratories from the aforementioned sites from July 2005 to December 2009 (objective 3), and July 2010 to June 2011 (objective 4). All entries in the database with information on susceptibility test results were used to analyse patterns of antimicrobial resistance.

2.4 Data Collection

For the retrospective study, data of blood culture isolates of selected pathogens were extracted from the Corporate Data Warehouse (CDW). The following variables were included: age, gender, geographical location, province, hospital name, year of entry, name of organism, antibiotics used for susceptibility testing and susceptibility results of each antibiotic tested. For the prospective study, surveillance in real-time required collection of the actual data as they became available during the year concerned. However, in this study data of blood culture isolates were obtained from the ARSR and contained all variables as outlined for the retrospective study above except year of test as data were collected only over a 1 year period.

2.5 Data Management

This part of the methods section is presented in three sections: Section 2.5.1 and 2.5.2 looks at how we managed the 2005 to 2009 blood culture data and section 2.5.3 deals with how the July 2010 to June 2011 blood culture data were managed.

2.5.1 Data extraction for 2005-2009 blood culture data

In this section we describe processes and procedures that were done, including programs used to extract data from the CDW and procedures describing how data were merged together to make a coherent single database in a flat file format.

Table 2.1 SQL statement programmed to extract data from the CDW

SQL Statement
--***** WO514421 Public Health 2005 to 2009 - changed to include bloods only 17th Nov****

select distinct t3.lab_no, t8.unique_patient_id, province, location_name, ward_name, tested_age_years, gender, specimen_type ,organism_name, drug_name,
 decode(sensitivity, 'R', 'RESISTANT', 'S', 'SUSCEPTIBLE', 'I', 'INTERMEDIATE') as sensitivity, taken_date, tested_date from target.fct_sensitivity_results t3, target.dim_organisms t2, target.dim_drugs t4, target.dim_locations t6, target.dim_patient_specimens t8, target.dim_dates t1
   where t3.organism_id = t2.dimension_key
   and t3.drug_id = t4.dimension_key
   and t3.location_id = t6.dimension_key
   and t3.lab_no = t8.lab_no
   and sensitivity in ('R', 'S', 'I')
   and trunc(t3.taken_date) = t1.day
   and reviewed_status_flag = 'REVIEWED'
   and health_sector = 'PUBLIC HEALTH SECTOR'
   and location_code in ('BAG', 'JOH', 'PRE', 'GRS', 'TYG', 'UNV')
   and organism_code in ('STAAU', 'KLEPN', 'PSEAE')
   and specimen_type in ('BLOOD', 'BLOOD AND CEREBROSPINAL FLUID', 'BLOOD CULTURE', 'FAN AEROBIC (BACT/ALERT BTL)', 'FAN ANAEROBIC (BACT/ALERT BTL)', 'MYCOLYTIC F (BACT/ALERT BTL)', 'PAEDIATRIC (BACT/ALERT BTL)', 'STD AEROBIC (BACT/ALERT BTL)', 'STD ANAEROBIC (BACT/ALERT BTL)', 'VENOUS BLOOD')
   and cal_year_number = 2005 --, 2006, 2007, 2008, 2009)
   order by province, t8.unique_patient_id, t3.lab_no

Table 2.1 highlights the exact SQL statement that was written and applied to the CDW repository to extract specific parameters of blood culture data required for analysis as outlined in section 2.5.1.2. Executing this query, blood culture data with specified parameters were extracted for each individual year 2005, 2006, 2007, 2008 and 2009, resulting in five data tables. For purposes of simplicity and comparison, these data tables were merged to form one single flat file. The step by step process of how five data tables were merged to form one single table for 2005 to 2009 is outlined below.

2.5.1.1 Combining NHLS Results Spreadsheets into One Microsoft Access Table

Step 1: All excel worksheets were imported as single Microsoft Access tables. They were named 2005, 2006, 2007, 2008 and 2009 respectively.

Step 2: A copy of the 2005 table was made and then renamed appropriately to "ALL_NHLS".

Step 3: An append query was create that appended the 2006 table to the 2005 table. The SQL view of the query "Append_2006" was appropriately viewed in access file.

Step 4: The append query was then run.

Step 5: Steps 3 and 4 were repeated for the 2007, 2008 and 2009 tables. The result was a single data table with all data values from 2005 to 2009.

2.5.1.2 Querying: Antimicrobial Susceptibility by Specific Pathogens

The query interrogated the database for records where the given pathogen, drug and resistance levels were available. Three queries were designed for the pathogens SA, KP and PA and as a researcher I needed to enter a specific antibiotic at run-time to get the relevant records for that particular medication that had undergone sensitivity testing. The following fields in excel spreadsheet were interrogated: i) organism_name, ii) drug_name, and iii) sensitivity.

2.5.2 Data cleaning procedure for 2005-2009 dataset

In this section we describe data manipulation and details of exclusion of duplicates as well as exclusion of records outside of the duration of the study.

Table 2.2 Processing of data after extraction from the CDW: 2005-2009 dataset

Sample size attrition	2005	2006	2007	2008	2009	Overall	Overall cleaned
Start sample	9,528	25,151	25,497	23,467	22,371	106,014	106,014
Following removal duplicates: patient id, organism, drug, taken date	8,087	21,946	21,535	20,257	19,546	91,371	91,371
Following reshape (long to wide - drug and resistance)	900	2,500	2,423	2,655	2,378	10,856	10,856
Remove repeat organism test for an individual within 21 days	744	2,120	2,055	2,238	2,068	9,225	9,218

The **start sample** was the number of records extracted from the CDW data base in long format, after applying the SQL program. The data was in long format hence the numbers of records extracted were extensively exaggerated. The exact number was, however, obtained after reformatting the data structure from long format to wide format; so that each record had all its parameters in a single row compared to multiple rows for a single test result. At the end, after excluding all duplicates as well as those which had repeated blood culture tests within 21 days. Seven more records from 2004 (9218 records) were obtained and carried over for further analysis.

Table 2.3 Stata output after adding omitted data from Helen Joseph hospital

```
Data long -> wide
-------------------------------------------------------------------------
Number of obs. 4880 -> 754
Number of variables 10 -> 43
j variable (35 values) drug -> (dropped)
xij variables:
r_ -> r_AMIKACIN r_AMPICILLIN ... r_VANCOMYCIN
-------------------------------------------------------------------------
tab year
      year |      Freq.     Percent        Cum.
-----------+-----------------------------------
      2004 |          3        0.03        0.03
      2005 |        812        8.14        8.17
      2006 |      2,294       23.00       31.18
      2007 |      2,209       22.15       53.33
      2008 |      2,393       24.00       77.33
      2009 |      2,261       22.67      100.00
-----------+-----------------------------------
     Total |      9,972      100.00
-----------+-----------------------------------
```

The first time the CDW data were extracted, blood culture data from Helen Joseph hospital were inadvertently omitted. Using a similar SQL program, data from Helen Joseph were

extracted and additional data (n=4880) were obtained. This data table was also restructured from long to wide format for drug profile and yielded 754 observations. This data was appended to the previous dataset with 9218 observations (Table 2.2, section 2.5.2) yielding a total of 9972 observations after ascertaining that no further duplicates were available in the dataset. Table 2.3 displays full dataset showing the yearly breakdown as well as appended dataset from Stata 12 outputs, after excluding 3 records from 2004, a total of 9969 records were analysed.

2.5.3 Data cleaning procedure for 2010-2011 blood culture dataset

This section provides a detailed outline of data cleaning and processing procedure of data that were extracted from the GERMS-SA database. We highlight details of exclusion of missing, non reference organism, as well as records outside of the study area and duration of the study. The procedure for data extraction for this particular dataset was the same as the one documented in section 2.5.1.

Table 2.4 Data cleaning and processing after extraction from the GERMS-SA database: June 2010 to July 2011 dataset

Sample size attrition	FS	GP	KZN	LP	WC	Overall	Overall cleaned
Start sample	407	4388	614	17	1520	6946	6946
Following removal LP & KZN*	407	4388	-	-	1520	6315	6315
Following removal of hospitals not included in the study**	407	4384	-	-	1513	6305	6305
Following removal if missing or organism not *Klebs/Staph*!!						6305	5004
Following removal if year was 2012 & organism not SA & KP!						5004	3026

*LP &KZN were not included in the study hence had to be excluded (631 observations omitted).
**Excluded data from hospitals not part of the study 30, 40, 72 (10 observations omitted)
!!Organism not Klebs or Staph species were excluded (1301 observations omitted)
! Reference organism not KP &SA, and tested outside the study period < July 2010 & > June 2011(1978 observations omitted).

The **start sample** of 6946 isolates in table 4 highlights the total number of records that were extracted from the GERMS-SA database and presented as a flat file in excel spreadsheet. The data were then transferred into Stata version 12 for cleaning and further processing. The number of isolates totalling 6946 included data from outside of the study period (July 2010 to June 2011), as such, the number of records appears to have reduced dramatically after cleaning had been completed. The number of 3026 isolates (SA=1494, KP=1545) was obtained after exclusion of observations from provinces and hospitals that were not part of the study as well as observations with missing data on organism name or organism belonging to the reference species (SA & KP). In addition, organisms that were neither *Klebsiella pneumoniae* nor *Staphylococcus aureus* (i.e. *K. oxytoca, K. terrigena, K. planticola, K. ozaenae)* were also excluded from the dataset. In the end, 631 observations from KZN and LP were excluded, 10 observations from non-study hospitals were excluded, 1301 observations with missing data on organism that did not belong to *Klebsiella and Staphylococcus* species were excluded, and 1978 isolates that were reported either before July 2010 or after June 2011 were also excluded. Therefore, a total of 3026 observations were carried over for further analysis of patterns of resistance and associated risk factors.

2.5.4 Assessing completeness of data & importation to Stata software

In this section we describe procedures followed for ascertaining reliability of data, cleaning data and importation of data to Stata for further analysis. Assessment of validity and reliability of the routine data was done by performing in-depth verification and quality analysis of available data from LIS. Missing information and errors on entry were investigated and assessed for completeness of data to be able to determine reliability of the data. As the opportunity to verify data with original data source was limited for the retrospective data, no attempt was made to do this. However, data that were entered

prospectively were interrogated to ascertain completeness through verifying missing data and uploading data directly from automated Micro Scan as a way of minimising incompleteness. The processing of data cleaning and verification of entries was done in MS Excel spreadsheets before exporting to Stata software version 12 (StataCorp Limited, College Station, Texas, USA) for detailed analysis.

2.6 Data Analysis

2.6.1 Descriptive Analysis

Descriptive analysis was done to show the distribution of characteristics of study sample and results presented as proportions in figures (histograms) and tables. All variables, including age, were categorized for subsequent analysis.

2.6.2 Unadjusted Analysis

A primary unadjusted statistical analysis was done using all entries in the database for the period 2005-2009. The crude resistance estimates were determined as well as trends and patterns of different antimicrobial agents for the three bacterial pathogens. We compared this with prospective data collected from July 2010 to June 2011. Bivariate cross-sectional comparisons were done using Pearson chi-square tests of independence for categorical variables. Associations were determined using univariate logistic regression analysis since the nature of the outcome variable was dichotomous.

2.6.3 Adjusted Analysis

Secondary adjusted analysis was conducted on potential confounding variables, such as age, gender, year, geographical location, hospital, and province. For binary variables, we

performed a chi-square test to compare differences in proportions between the two groups. Since the outcome variable was dichotomous (Resistant: Yes, No), adjusted comparisons were done using multiple logistic regression to account for covariates. Variables that were associated with the outcomes were selected by a backward elimination regression analysis. A likelihood ratio test with p≤ 0.05 significance level was used to compare the fit of different models, one of which was nested within the other. This was done to assess if a simplified assumption for a model was valid. The odds ratios were calculated against the reference values of the outcome variable. Odds ratios were reported to show associations between resistance and different covariates. Resistance rates were compared across age-groups, gender, geographical regions (provinces), hospitals and year of infection with the assumption that the null hypothesis showed that there was no association between provinces, hospitals, year, and resistance rate. The significance level was predetermined at 0.05 with 2-tailed *P* values.

2.7 Ethical Consideration

The protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand (approval number M10625). This study was part of a larger National Institute for Communicable Diseases (NICD) surveillance programs that obtained ethical clearance for ARSR and GERMS-SA to conduct communicable diseases surveillance.

2.8 Limitations of the study

The major limitation of this study as a whole was incompleteness of microbiological data, an inherent problem with surveillance systems. Also differences in quality of susceptibility testing between different participating laboratories were obstacles for reliable comparison of data originating from various laboratories.

2.9 Dissemination of findings

The findings of this study were disseminated to a wider scientific community through publications in peer reviewed journals and presentations at national and international conferences. The published and unpublished papers together form the basis for this doctoral thesis.

Chapter 3   Systematic Review of Published Literature: Antimicrobial Resistance Surveillance among Nosocomial Pathogens in South Africa

This chapter gives details of the findings of a systematic review of published literature on antimicrobial resistance in South Africa. The findings have been published in a paper entitled **"**Antimicrobial Resistance Surveillance among Nosocomial Pathogens in South Africa: Systematic Review of Published Literature", *Journal of Experimental & Clinical Medicine,* Volume 4, Issue 1, Pages 8-13. Jan 2012 (Appendix 12.3).

3.0 Abstract

Background: There has been significant increase in the prevalence of antimicrobial drug resistance in sub-Saharan Africa. This may increase health care costs due to patients' need for more diagnostic tests, longer hospitalization and poor outcome. Therefore, monitoring systems for resistance patterns are needed to effectively minimise poChapter 4or outcome. A systematic review was conducted to find out the prevalence of antimicrobial drugs resistance among S*taphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and to understand whether or not such data was part of an ongoing surveillance system for nosocomial infections in South Africa.

Method: An online search of main databases including Cochrane Library, PUBMED and MEDLINE was done using search terms: "antimicrobial resistance" and "surveillance", "antimicrobial susceptibility" and "surveillance" or "*Staphylococcus aureus*" or "*Klebsiella pneumonia*" or "*Pseudomonas aeruginosa*"; "nosocomial" or "hospital acquired", "South

Africa" or "Africa". We also did manual search of local conferences, theses and dissertations to identify relevant articles.

Results: In total, 41 manuscripts were identified of which eight were analyzed. There is no evidence of ongoing antimicrobial resistance surveillance for nosocomial pathogens in South Africa. Data reported in this review seem to have been analysed on ad hoc basis and do not show a particular resistance pattern, however, data shows evidence of resistance to commonly used antimicrobial drug in this population: for *S. Aureus* resistance to cloxacillin was 29%; and 38% to erythromycin; for *K. pneumonia* resistance to ciprofloxacillin was 35% and 99% to ampicillin; and for *P. aeruginosa*, the mean resistance to ciprofloxacillin was 43% and 35% to amikacin.

Conclusion: Surveillance of antimicrobial resistance is essential to better understand the complexity of antimicrobial resistance development. Such evidence would be used in developing an effective surveillance programme to monitor patterns and trends of resistance overtime.

3.1. Introduction

Antimicrobials are essential for the treatment of infectious diseases. However, a high prevalence of resistance impacts patient outcomes negatively. Antimicrobial resistance increases health-care costs due to a need for more diagnostic tests, additional drugs for treatment, and longer duration of hospitalisation. (12, 32) Therefore, the emergence and spread of antimicrobial-resistant organisms from hospital to the community, is a growing public health challenge in South Africa and worldwide. Antimicobial resistance is associated with a high level of morbidity and mortality, and for this reason, antimicrobial resistance requires effective monitoring to determine patterns and trends over time. (83-86) For South Africa, such information is particularly important because of the HIV/AIDS epidemic and increased antimicrobial consumption due to frequent episodes of opportunistic infections.

Antimicrobial resistance surveillance is crucial for evaluating the use of empirical antimicrobials for treatment. (26) Continuous monitoring and a better understanding of the profile and magnitude of antimicrobial resistance are therefore required. This will help address the problem of increasing rates of antimicrobial resistance in South Africa. The European Antimicrobial Resistance Surveillance System (EARSS) is an electronic laboratory information system that has been used as a tool for identifying emerging antimicrobial resistance. (87) In South Africa, an equivalent national surveillance system to monitor the status of antimicrobial resistance for nosocomial pathogens has not yet been established. For this reason and as an interim exercise, this review was initiated to gather scientific evidence of the extent and patterns of antimicrobial resistance in selected hospital-acquired pathogens in South Africa.

3.2. Methodology

3.2.1 Online Search Strategy

A comprehensive search of biomedical databases was carried out to find all relevant manuscripts published in English. The search aimed at identifying relevant peer-reviewed epidemiological studies that would provide adequate information on antimicrobial surveillance initiatives in South Africa.

3.2.2 Search engines, dates of publications and search words used

The following search terms were used: "antimicrobial resistance' and "surveillance"; "antimicrobial susceptibility" and either "surveillance" or "*Staphylococcus aureus*" or "*Klebsiella pneumoniae*" or "*Pseudomonas aeruginosa*"; "nosocomial" or "hospital acquired" or "South Africa" or "Africa". We focussed on searching pathogen-specific literature and data for this review using manuscripts identified through an extensive search of the following databases: Cochrane Library (July 2011); MEDLINE (1966 to July 2011); African Journal on line (AJOL) (1980 to July 2011), EMBASE (1980 to July 2011); and LILACS (1982 to July 2011) on www.bireme.br.

3.2.3 Manual Search Strategy

We also carried out a manual search and review of the reference lists of the identified articles. Additionally, as findings of studies are not always published conventionally, we manually searched the abstracts and proceedings within the past 10 years of the following conferences: "OIE International Conference on Antimicrobial Resistance", "Conference on Antibiotic Resistance Prevention and Control" (ARPAC), "Public Health Association of Southern

Africa" (PHASA), "Federation of Infectious Diseases Society of South Africa" (FIDSSA),

"Global Antimicrobial Resistance Program" (GARP), "Congress European Society of

Clinical Microbiology and Infectious Diseases" (ESCMID), and the "Congress of the

International Society for Infectious Diseases". Such conference proceedings outline major

group sessions for microbiology and infectious diseases specialists working within the field

of antimicrobial resistance. We did not obtain any relevant data from these searches. In

addition, informal approaches were made to individuals and organizations within the field of

hospital infection control and antimicrobial resistance surveillance for information regarding

unpublished data, dissertations and theses.

This search yielded four of the 8 papers that were included for analysis. Data for rates of

antimicrobial resistances were presented as means.

3.3 Results

3.3.1 Antimicrobial resistance surveillance for invasive pathogens in South Africa

A good surveillance system for antimicrobial resistance monitoring should involve ongoing

collection and collation of both clinical and microbiological data, with an emphasis on

timeliness, accuracy, consistent and standardised methods of collection and analysis, using a

centralised laboratory with appropriate control measures focused on reporting on nosocomial

pathogens. Such a system has not been established in South Africa. However, although

different methods were used, they were all approved by the National Committee for Clinical

Laboratory Standards (NCCLS), predecessor of the Clinical Laboratory Standards Institute,

and therefore suitable for trend analysis e.g. ciprofloxacin resistance in *K. pneumoniae*

increased in academic hospitals from 18% (24/1324 isolates in 1990) to 28% (498/1778

isolates) in 2007.

From the included studies, lack of clinical data and quality assurance information are deficiencies requiring attention; nonetheless, some steps have been taken to contain resistance development. Prudent use of antimicrobial (antimicrobial stewardship) has been looked at through the South African Antibiotic Stewardship Programme (SAASP) while the South African Society of Clinical Microbiology formerly the National Antimicrobial Surveillance Forum (NASF), focuses on AMR surveillance and reporting using passively collating antimicrobial data in public hospitals through the National Health Laboratory Services (NHLS) and in private health- care sectors through private microbiology laboratories.

The Antibiotic Study Group of South Africa has been active since 1976 (88), this group joined public sector surveillance in 2002 as NASF, meeting and sharing information, and several publications in the area of antimicrobial resistance has been released. (88-91) More recently, the Group for Enteric, Respiratory and Meningeal Diseases Surveillance (GERMS-SA), an established entity within the National Institutes for Communicable Diseases (NICD), has been established, which operates in all nine provinces, focussing on surveillance of community-acquired pathogens and monitoring resistance profiles.

As of 2010, a surveillance to monitor resistance among *S. aureus and K. pneumoniae* was established as part of GERMS-SA. Another initiative was introduced in KwaZulu Natal for surveillance of *E. coli* in 2000/2001 (65) and the Veterinary Surveillance of Antimicrobial Resistance in South Africa has been involved in monitoring resistance among zoonotic infections. (66) Table 3.1 illustrates hospitals and laboratories that contributed antimicrobial susceptibility data for the studies that were included in this review.

Table 3.1 Public and private sector laboratories that participated in antimicrobial susceptibility data over the period 2000-2011

Public Sector Hospitals/NHLS laboratory*	Private sector laboratories ^^
Chris Hani Baragwanath Hospital	Drs Bouwer & Partners (Ampath)
Charlotte Maxeke Johannesburg Academic Hospital	Drs Dietrich & Voigt (Pathcare)
Steve Biko Academic Hospital	Drs du Buisson, Bruinette & Partners (Ampath)
Dr George Mukhari Hospital	Drs Mauf & Partners (Lancet)
Pelonomi & Universitas Hospital	Drs Swart & Marais (Ampath)
Groote Schuur Hospital	Drs van Rensburg Pathologists
Tygerberg Hospital	Drs Vermaak & Partners
Green Point NHLS Laboratory	Niehaus & Botha
King Edward VIII	
No. 1 Military Hospital	

NHLS=National Health Laboratory Service

* NHLS from Gauteng Province (Johannesburg, Pretoria), Free State Province (Bloemfontein), KwaZulu Natal Province (Durban and Western Cape Province (Cape Town). ^^ Private laboratories in Gauteng Province (Johannesburg, Pretoria), KwaZulu Natal Province (Durban), Western Cape Province (Cape Town) and Free State Province (Bloemfontein)

3.3.2 Description of study settings and study designs [27, 91-98]

A total of 41 manuscripts were identified: 26 identified through database searches and 15 through manual searches in libraries and among personal contacts. Twenty-three were excluded as they did not meet the criteria for inclusion which was full text articles with antimicrobial susceptibility data reported from multiple sites. This left behind 18 that had full-text article reviews to further assess for eligibility, and 10 more were further excluded. Eight manuscripts published between 2000 and 2011 were identified and included in this review (Figure 3.1).
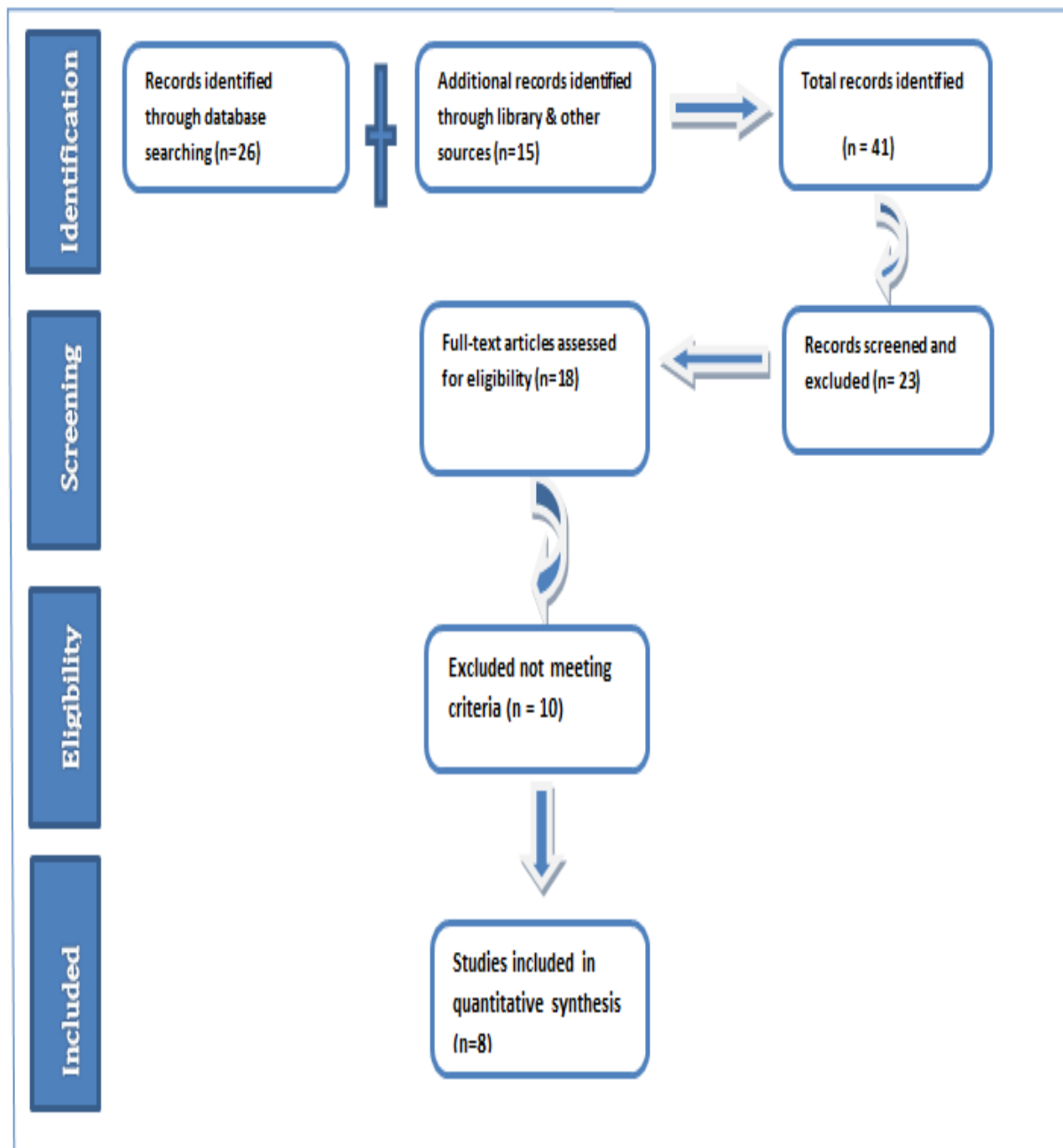
Figure 3.1 Flow diagram of antimicrobial resistance studies included in the review. Note, from PRISMA: www.prisma-statement.org.

Of the eight manuscripts, five were published prior to 2007. All manuscripts identified for this review included susceptibility data from only four of the nine provinces of South Africa. Five of these studies were from public sector tertiary hospitals and three were from private sector laboratories, predominantly from urban settings across South Africa. (Table 3.2)

Seven of these studies produced results from surveillance data aggregated from more than seven sites nationwide, while one study produced results from surveillance data from 16 hospitals within KwaZulu Natal province. None of the eight studies detailed the study design used, other than stating that the study was "multi-site and used data of blood culture isolates from microbiology laboratories". Only one study used isolates from respiratory aspirates. (96) All except one study from various public sector hospitals within KwaZulu Natal Province used retrospective laboratory data. (97) (Table 3.2)

Table 3.2 Characteristics of antimicrobial resistance studies in South Africa

Author	Year	Pathogen	Location	Sample Type	Source of information	Study Design
Bamford et al.[87]	2009	SA, KP, PA & others	8 NHLS Laboratories	Blood & CSF	NHLS Surveillance data	Not specified
National Antibiotic Surveillance Forum[92]	2008	SA, KP, EC & others	Private labs, number of labs involved not mentioned	Blood & Urine	Private laboratories data	Not Specified
Brink et al.[22]	2007	SA, KP, PA & others	7 Private laboratories	Blood	Private laboratories data	Not specified
Sein et al.[89]	2005	SA, KP, EC & others	7 NHLS laboratories	Blood & CSF	NHLS Surveillance data	Retrospective approach
Essack et al.[91]	2005	SA, KP, PA & others	Laboratories in 16 hospitals	Blood	Public sector surveillance data	Multicentre Study in RSA
Liebowitz et al.[90]	2003	KP & others	12 Private laboratories	Sputum, bronchial brush, BAL, pleural fluid, sinus tap, MEF, pharyngeal swabs	Private labs data	Multicentre study in RSA
Crewe-Brown et al.[88]	2001	SA, KP, EC & others	8 NHLS Laboratoriess	Blood & CSF	Public sector surveillance data	Not specified
Antibiotic Study Group.[85]	2000	SA, KP & others	8 NHLS Laboratories	Blood & CSF	Public sector surveillance data	Not specified

BAL= bronchial alveolar lavage; CSF=cerebral spinal fluid; EC= *Escherichia coli;* HI=*Haemophilus influenza*; KP= *K. pneumoniae*; MEF=middle ear fluid; NHLS= National Health Laboratory service; PA=*P. aeruginosa;* SA=*S. aureus;* SA= Republic of South Africa; SP= *Streptococcal pneumonia*

3.3.3 Description of microbiological methods [27, 91, 93-95, 98]

Seven of the studies were conducted using data from blood and cerebral spinal fluid (CSF); (27, 91, 93-95) one study used data from respiratory aspirates and urine. (98) The methodologies of antibiotic susceptibility testing were described in seven studies, all of which mentioned the use of the CLSI breakpoints formerly NCCLS to determine antimicrobial susceptibilities. Two studies described in detail other methods used for susceptibility testing of various antibiotics, such as Kirby-Bauer disk diffusion, Broth micro dilution, E-test and use of automated Vitek 2 system. (27, 96) Only one study mentioned quality control in identification and susceptibility testing as per CLSI recommendations. (27) All studies used only one sample per patient hence duplicate samples were excluded to minimize over-representation of the cases that had multiple and frequent cultures. Two studies that reported antimicrobial susceptibility of respiratory tract pathogens, mentioned intermediate- and high-level resistance for such organisms. (94, 96)

3.4 Resistance rates for different pathogens

3.4.1 *Staphylococcus aureus* [27, 91, 93-95, 97, 98]

Susceptibility data for *S. aureus* were reported in seven studies. (Table 3.2) Five of these studies were from public sector laboratories and two studies from private sector laboratories.(91, 93-95, 98) Geographically, all studies identified were performed in urban areas except one study done in Durban, which included isolates from district and regional hospitals. Specimen types included blood and CSF, except one study that included respiratory aspirates. (Table 3.2) The resistance rate of *S. aureus* to cloxacillin was 29% (range 23-69%); erythromycin 38% (25-46%); gentamicin 20% (range 7-45%), and methicillin resistance (MRSA) was 33% (17-59%). As much as cloxacillin resistance is synonymous for MRSA

there were differences in the way resistance was reported in the papers included in this review. No resistance to linezolid has been reported since its introduction in 2000, while frequency of resistance to glycopeptides was uncertain due to disagreement on optimization of vancomycin susceptibility testing. (Figure 3.2)
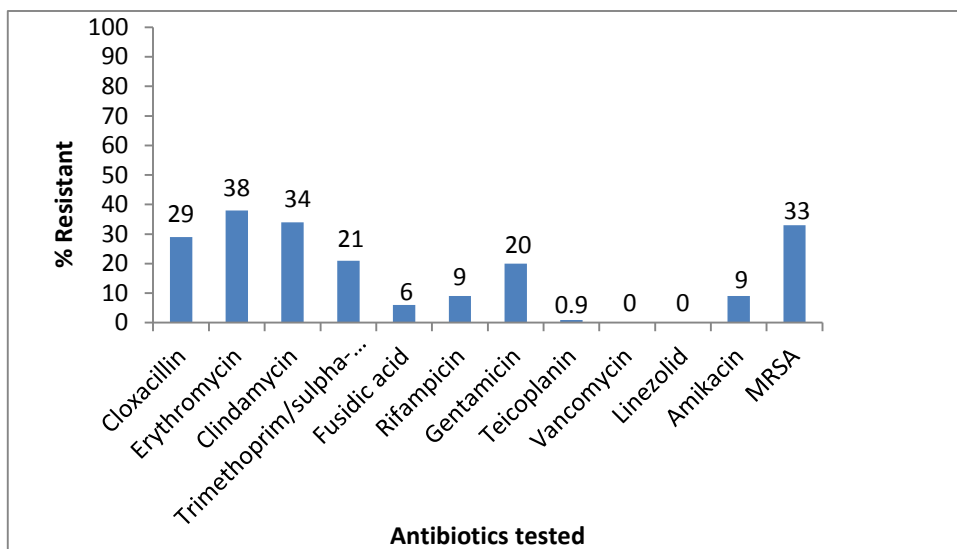
Figure 3.2 Proportion of antimicrobial resistance among *S. aureus.*

Note: Data were from seven published studies, between 2000 and 2009.* Different methods were used to determine MRSA status (Cloxacillin resistance of 29% vs. 33% MRSA, there was no record for cefoxitin screening for MRSA which might explain the difference)

3.4.2 *Klebsiella pneumoniae* [27, 91, 93-98]

Most studies that reported on susceptibility patterns for *Klebsiella pneumoniae* were published by the Antibiotic Study Group that used data mostly from large public sector academic hospitals that provide services to a diverse population group. Clinical isolates were predominantly from blood & CSF culture (four studies), blood culture only (one study), blood & urine culture (one study) and respiratory aspirates (one study). The resistance of *K. pneumoniae* to ciprofloxacin was 35% (range 15-65%), cefuroxime 52% (range 27-72%), gentamicin 50% (range 18-70%) and ampicillin 99% (range 88-100%) (as expected, as all KP

carry *bla*_{SHV}gene). Resistance was almost non-existent for imipenem, meropenem and moxifloxacin. (Figure 3.3)
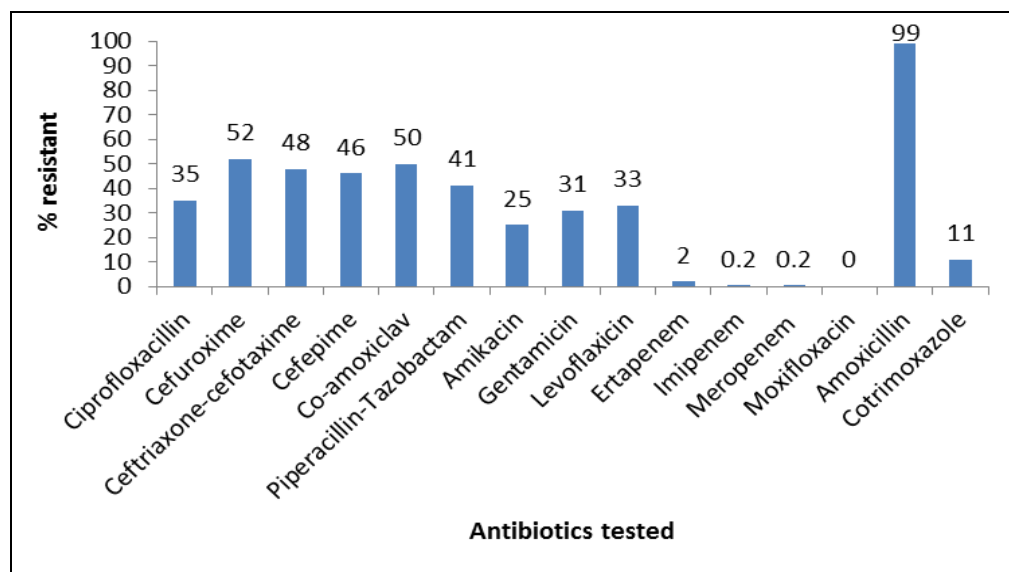
Figure 3.3 Proportion of antimicrobial resistance among *K.pneumoniae*. Note: The data were from eight published studies from 200-2009

3.4.3 *Pseudomonas aeruginosa* [27, 93, 97]

Three studies reported resistance rates for *P aeruginosa*, two of which were from blood culture isolates and one from non-specific sources.(27, 93, 97) The resistance among *P. aeruginosa* to ciprofloxacillin was 43% (range 30-75%), gentamicin 50% (range 10-65%), amikacin 35% (11-67%) and aztreonam 42% (range 25-75%). Resistance to polymyxin was <5% (range 0-5%) and was reported in a single study. (27, 93, 97) Resistance rates to almost all drugs tested were greater than 30%. (Figure 3.4)

A study done by Perovic et al using data from 1998 to1999 at Chris-Hani Baragwanath hospital showed that there was an association between *P. aeruginosa* bacteraemia and outbreaks caused by multiple-resistant genotypes. In this study, the proportion of nosocomially-acquired infection was 57.1%. (99) The resistance profiles and incidence of disease are likely to have changed during the 10-year period, and the current status may be

different but is unknown. This review shows high resistance rates of *P. aeruginosa* to most

conventional antibiotics.
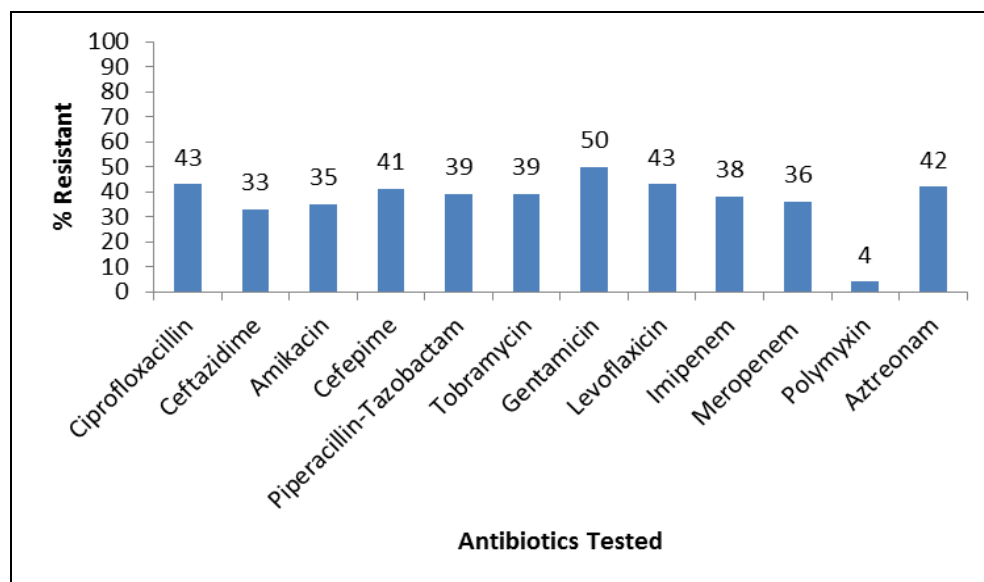
Figure 3.4 Proportion of antimicrobial resistance among *P.aeruginosa*. Note. Data were
from three published studies from 2005-2009

3.5 Presence of Extended-Spectrum Beta-Lactamases (ESBLs)

Seven studies included in the review reported on extended spectrum beta-lactamases (ESBLs)

in *K. pneumoniae*. In academic hospitals, the rates of ESBLs increased from 33% (436/1324)

in 1999 to 49% (869/1778) in 2007. These studies used the double-disk method and reported

resistance rates as high as 59% and 62% in private hospitals and public sector hospitals,

respectively. A study done by Sabiha Essack at a teaching hospital in Durban between 1994

and 1996 investigated ESBL-mediated resistance in South African nosocomial origin of *K.

pneumoniae* and demonstrated that each of the isolates expressed 1-6 β-lactamases. (100)

3.6 Discussion

This systematic review assessed the literature about the prevalence of resistance to commonly used antimicrobials as well as whether or not such data were part of an ongoing surveillance system for nosocomial infections in South Africa. We found that no national surveillance system existed that collected and collated data year on year, to assess trends and resistance pattern for nosocomial pathogens. In addition we found a high overall proportion of resistance to antimicrobials used for empirical treatment.

Except for resistance to polymixins which is very uncommon in *P. aeruginosa* isolates, resistance rates to other antimicrobials commonly used for the treatment of infections caused by this bacterium are high. The study found a low level of resistance among *Klebsiella pneumoniae* to moxifloxacin and carbapenems and a pattern of high resistance to other classes of antimicrobials that are commonly prescribed. *S. aureus*, showed no resistance to teicoplanin, vancomycin, and linezolid, but high resistance to other classes of antimicrobials. This is similar to resistance pattern in Central African countries, as shown in a review by Vlieghe et al, (101) even though their study focussed mostly on community acquired pathogens.

Several limitations have been observed in this study: Firstly, studies included in this review reported laboratory data on antimicrobial-resistant isolates with no clinical data; hence, they could not link resistant isolates to clinical findings. Secondly, most studies included in this review aggregated data from different laboratories which employed varied laboratory techniques. This was not ideal for surveillance purposes but all methods were NCCLS/CLSI approved. Thirdly, data used were collected retrospectively, except for a single study by

Brink et al that collected data prospectively. (27) Use of retrospective data has several limitations, including incomplete data that are subject to numerous biases. Fourthly, most if not all, studies lacked demographic data; hence, it was difficult to compare community-acquired versus hospital-acquired infections. Lastly, variation in clinical specimens, taking practices between different institutions might alter consistency and comparability of data reported from these various studies. Furthermore, this study included invasive pathogens from blood cultures as well as pathogens from respiratory specimens and, in the case of *P. aeruginosa,* also from other sources, including burns. It should be noted that blood culture isolates are highly predictive of truly invasive disease and unlikely to be contaminants. It is true that resistance may first arise from infections from other sites including skin and intestinal tract. Blood culture isolates that are resistant to antibiotics are very likely to be virulent while cultures from other sites where colonization occurs, the resistant organisms may be less virulent and harbour resistance mutations which arise at a fitness cost and therefore less likely to be invasive.

In spite of the limitations mentioned above, there is growing evidence of escalating rates of antimicrobial resistance to several conventional antimicrobials. Even though vancomycin resistance is still negligible, ESBL and MRSA rates are high in these urban academic centers and private institutions. This is consistent with growing evidence of global trends of antimicrobial resistance reported previously showing a significant increase in of incidence of cefotaxine-resistant *Acinatobacter* infections as well as antimicrobial resistance in common bacteria health care associated pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* among others. (102, 103) Recent published data shows that rate of resistance is rapidly growing in China (22% average growth over six years, 1994 to 2000), Kuwait (17% average growth over four years, 1999 to 2003), and the U.S (6% average growth over three years, 1999 to 2002). (104)

This emphasizes the fact that surveillance is essential to further our understanding of antimicrobial resistance development and how it relates to prescription practice. (100, 101) Such undertaking will pave the way for designing interventions that could overcome resistance development to established antimicrobial agents.

3.7 Conclusions

Evidence suggests that antimicrobial resistance rates to nosocomial pathogens are generally high in South Africa. This is an emerging threat to public health and clinical management of patients with such infections in the face of dwindling antimicrobial development. We believe that a good surveillance system would enhance effective monitoring of emerging resistance and changes in resistance profiles, and identify significant differences in trends and distribution of antimicrobial resistance.

Chapter 4: Laboratory Information System: A surveillance tool for monitoring trends and patterns of resistant strains of important nosocomial bacteria

This chapter highlights findings of a critical analysis of the utility of the laboratory information system (LIS) of the National Health Laboratory Services (NHLS). The aim was to investigate the effectiveness of LIS in capturing reliable antimicrobial resistance data of important nosocomial bacteria i.e. *S. aureus*, *K. pneumoniae* and *P. Aeuginosa*, isolated from tertiary public hospitals in South Africa. This chapter has two sections: i) section 4.1 'The role of laboratory information system in antimicrobial resistance surveillance; ii) section 4.2 Laboratory methods and its impact on antimicrobial resistance surveillance.

4.1 The role of a Laboratory Information System in antimicrobial resistance surveillance

4.1.1 Introduction

The laboratory information system is a technique that the laboratory uses to deliver accurate and understandable results to the clinician who requested the analysis within a reasonable timescale. The system entails a sequence of events which includes 'transferring of a sample to the laboratory, analysing the sample, checking results or reanalysing the sample and releasing results to the clinician who requested the test'. In short, the concept of LIS refers to the computerised laboratory system or automation of clerical labour-intensive activities associated with the processing of laboratory results to improve accuracy and turnaround time of results. Automation of laboratory activities removes the element of manual reporting and allows access to retrospective data for analysis. (105) Previous studies have reported an improvement in the accuracy of data and turnaround time of laboratory results after installation of the LIS.

Therefore, due to the complex and large volume of data that these laboratories manage, and the continued demand for data to aid public health surveillance for effective disease prevention, there is a need for an operational LIS that can efficiently integrate and handle all sophisticated processes and procedures related to data from different laboratory departments including, but not limited to, microbiology, parasitology, virology, histopathology, biochemistry, haematology, endocrinology, cytology, toxicology, serology, immunology etc. (106, 107) Data for all such activities aggregates at the corporate data warehouse (CDW)

repository. This data could be used to measure sample volumes, estimate revenue, turnaround time, budgeting; monitoring antimicrobial resistance etc.

4.1.2 General description of LIS components and function (related to Figure 1)

A laboratory information system as illustrated in Figure 4.1 below constitutes the following components: hardware (computer system), software (computer programs), human capital (people who order laboratory tests, transports samples, etc.), laboratory procedures (i.e. blood culturing, susceptibility testing etc.) and data (laboratory results).
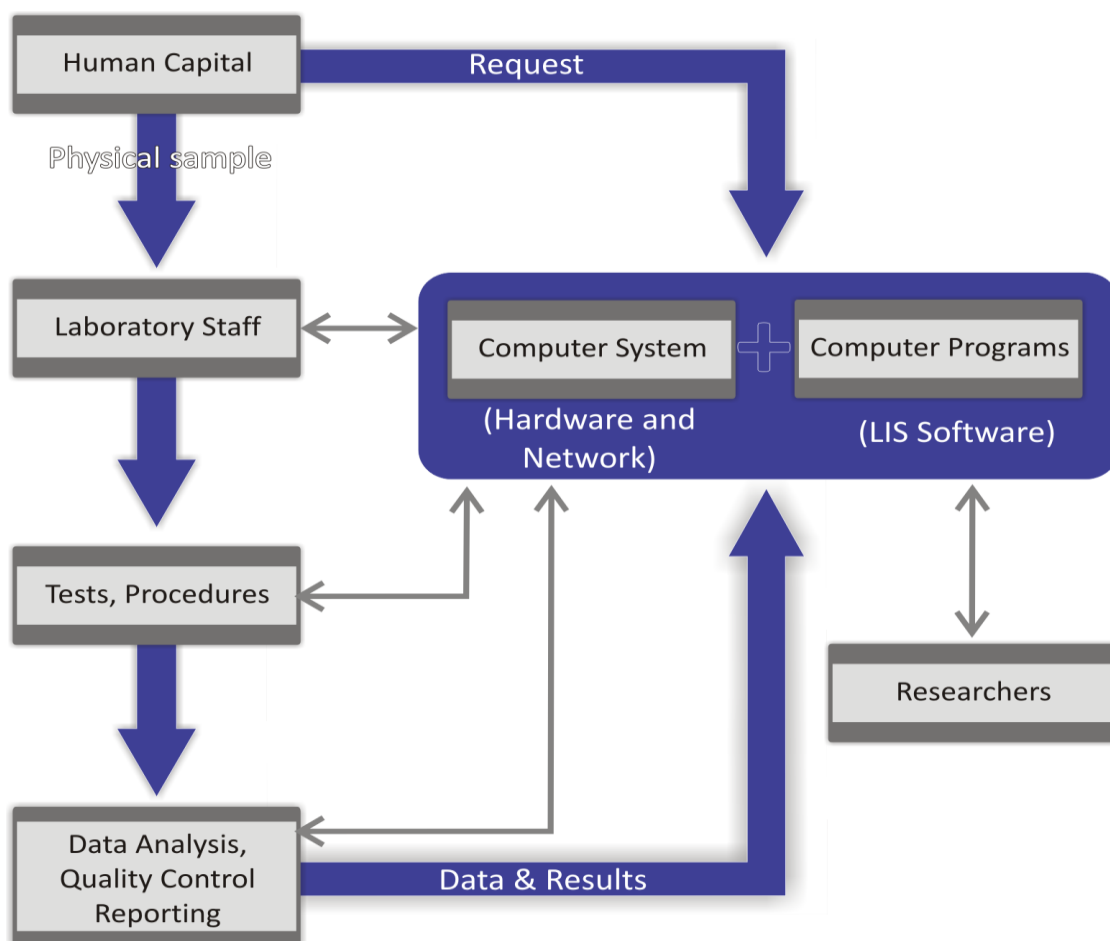
Figure 4.1 The Components of a Laboratory Information System

These components support each other interactively in the collection, processing, storage, distribution of data obtained during laboratory procedures. The system simplifies the process of tracking and sorting laboratory data, improves turnaround time of laboratory results and allows retrospective analysis of data for surveillance or research purposes. (54) Figure 4.2 below describes diagrammatically the processes and procedures in the function of a LIS.
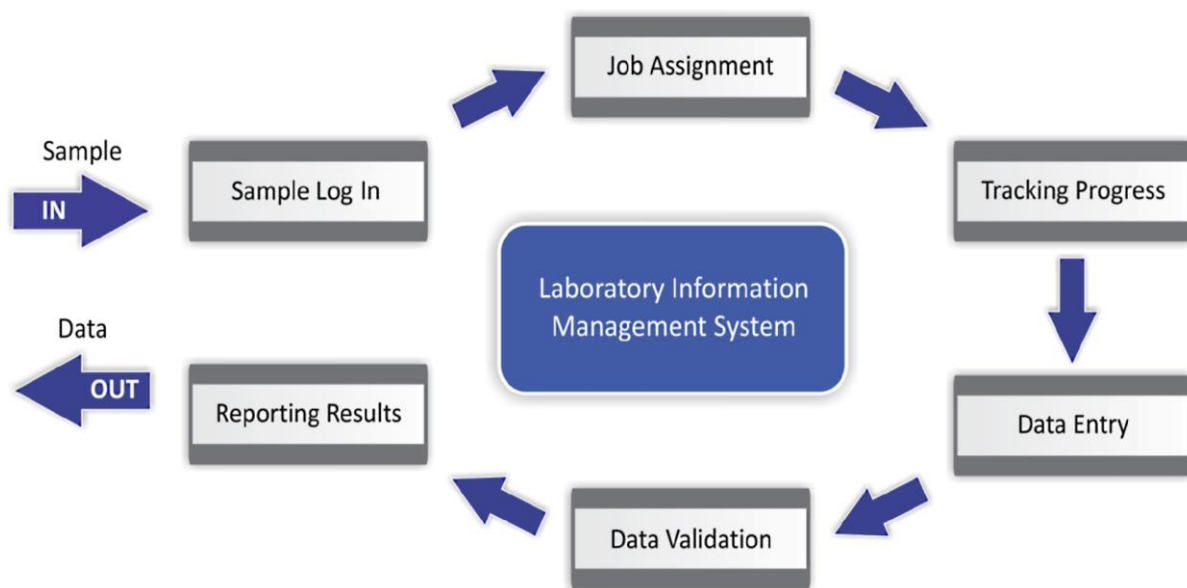
Figure 4.2 Flow chart showing Laboratory Information Management System processes and procedures (108)

The LIS is a complex system that simplifies and improves efficiency of laboratory operations, minimise data entry errors and deliver valid and reliable laboratory results to the patient and clinician in the most efficient way. In addition the system provides a platform for surveillance such as monitoring antimicrobial resistance following utility of retrospective data archived in the LIS database. (54) In South Africa, the NHLS LIS operates on software supplied by the Laboratory System Technologies (Pty) Limited, known as DISAlab.

4.1.3 The National Health Laboratory Services (NHLS)

The NHLS is mandated by the National Department of Health to provide public health laboratory services throughout South Africa. The aim is to deliver laboratory data to support clinical decision making within a reasonable and acceptable time so as to achieve good clinical outcomes among the patient population, and to attain improved laboratory performance and communication with clinicians. The NHLS provides laboratory and related public health services to over 80% of the population through a national network of over 300 laboratories, which is an integration of state owned laboratories: laboratories of the National Institute of Communicable Diseases (NICD) and the National Institute of Occupational Health (NIOH). These laboratories provide services to all public clinics and hospitals.

The process of accreditation of the laboratory allows for checking and assessing of errors, and acceptable limits are always incorporated into the system. As a researcher, it is advisable to apply acceptable standards to the data so as to account for any margin of error i.e., 5% error margin. For internal quality control measures, the LIS has check points to minimise data errors, but also validates data entries. In the event of failed validation, verification of data entered is done manually by another laboratory member of staff. For external quality control, there is a process of external quality assurance/quality control (QA/QC) that happens in all NHLS laboratories. This process is described in greater detail in section 4.2.

The NHLS DISA LIS, which has been in use for several years, is now migrating to TrakCare Lab, which is currently being rolled out in all the 322 laboratories by Health Systems Technology. (107, 109) Processes and procedures of the operations of the LIS will be

discussed in this section, drawing a parallel between the outing of DISA Lab and the incoming of TrackCare, weaknesses and strengths will be highlighted and suggestions for improvement of the LIS in the context of its role, as a tool for surveillance of antimicrobial resistance will be highlighted.

4.1.4 The DISALab LIS

The DISALab is a laboratory information system that was developed and maintained by Laboratory System Technologies (Pty) Ltd. The NHLS adopted the DISA LIS which has been in use for over 20 years.

4.1.4.1 DISALab System Modification

In response to the growing demand for accurate laboratory billing as well as understanding the volume of laboratory specimens sent for analysis in the public microbiology laboratories, and the provision of reliable laboratory data, the system has undergone several software modifications. Such modifications were in line with changes in the Information Technology (IT) industry as a result of developments of new software programs etc. Since its inception, DISA has of May, 2013 run more than 420 upgrades to the original program.

The DISALab system, that NHLS has been using over the past two decades has a built-in function that automatically updates the records once data is entered, hence each time a record is entered on the registered particulars, all corresponding records get updated automatically. Any change that happens to a particular record gets an automatic update of that record. Information is also recorded on the system to be able to capture who entered the changes into the system and at what time were such changes made. The IT managers for each laboratory

were able to alter certain segments of the system to suit the needs of the local laboratory, as such all changes taking place at each site by the IT personnel were noted at the DISA head office. Such changes led to an improvement in the functioning of the DISALab system.

4.1.4.2 The DISALab Hardware and Software System

All NHLS laboratories use automated equipment for incubation of the blood culture specimens. The BACT ALERT 3D (BA-3D) is the most commonly used blood culturing machine in NHLS microbiology laboratories. These machines function on a continuous basis, as new samples are loaded one after the other. The BA-3D machines are linked to the user interface, which transmits all the data electronically from the source to the user computers i.e. in clinics and hospitals where results are accessed electronically. Only relevant data from the BA-3D machine are selected and delivered to the central data point (data server). DISALab system is accessible at https://labresults.nhls.ac.za and using a drop down menu, the user is requested to select the province of operation; the system will then ask for the log in details.

The DISALab, a password protected system, was installed as a local system without inter-hospital connectivity; however a joint interoperability function had been established, since all data from different NHLS laboratories converged and aggregated at the central NHLS server in Johannesburg. The central data point was interlinked with individual servers located in different NHLS laboratories in all provinces.

All NHLS laboratories in each province routed data into a local provincial server only. From the local server data is transferred automatically to the central repository 'the corporate data warehouse' (CDW) at the NHLS headquarters in Sandringham, Johannesburg. For this reason, individuals who want to access at results from the DISALab website may only be able

to access results from a particular geographical area as the connectivity is not in real time, but also, that each province feeds data into a local server.

The DISALab LIS operates on the Open DataBase Connectivity (ODBC) system, which is an open standard application programming interface (API) for accessing a database (107, 110) and allows upgrades, which are often necessitated by frequent advancement occurring in the overall information technology industry. (111)

4.1.4.3 LIS quality control mechanism

The DISALab LIS has some built-in quality control mechanisms. The interface is validated continuously, to ensure that accurate information is transmitted to the central repository each time the scheduled data transfer procedure takes place. This helps to check for errors and confirm if the results produced are as expected.

The LIS is programmed to provide information off hand on the laboratory staff that performed a particular procedure and the time of the day. The system has built in memory that records all information pertaining to the user or an individual that entered the information. In the event that an error occurred, it is possible to identify the source of the error observed on the database, hence it is possible to rectify the error, but also minimise the possibility of erroneous reporting of results occurring from simple human mistakes.

The variable time in the LIS database needs to be emphasised as it is an essential component of quality control. For this reason, time is indicated for each activity on the laboratory request form. This variable 'time' helped, to a great extent, to track down the source of any error as

the laboratory in-charge would be able to identify the laboratory staff responsible for the procedure and the time of the day when the error occurred.

Parameters entered into the LIS database are the following: Sample ID, time collected, time registered, demographics such as date of birth or estimated age, gender, hospital, ward, province, clinical diagnosis (scanty data), organism cultured, drug sensitivity ((resistant/sensitive (R/S); minimum inhibitory concentration (MIC)), date sample was first registered and tested, date and time results were reviewed, instrument used for testing, sensitivity of antimicrobials, date clinician printed the results and the first laboratory where specimen was taken. If the sample was referred, then the results are only collected from the referring laboratory and not the laboratory where the test was done, as such, delays in getting the results might occur in the process.

4.1.5 Data Flow from the laboratory to the CDW

The blood culture results were entered into the LIS data base by the laboratory technologist as previously described. The data were then transmitted to a local laboratory repository prior to being relayed to a central repository. The first step that takes place in the laboratory is essentially immediately, while the second step may take up to 6 - 24 hours to be completed. At the CDW, data is processed 4 times per day and due to the fact that data is not in real time with the current DISA-Lab LIS, report generation can be difficult since data need to be extracted first and then processed.

The system is highly fragmented and data is processed in batches, coming from approximately 13 repositories in the 8 provinces. The rate of blood culture data flow between entry of data, laboratory storage and the central repository depends on the volume of data at

each point in time and the technology (network) involved. In tertiary academic hospitals, data movement takes relatively less time from one point to the other, compared to other facilities.

The CDW houses data repositories from all laboratories within the NHLS that are interlinked to the DISALab LIS, including microbiology data from blood cultures, cerebral spinal fluid cultures, stool and urine cultures, pus swabs among others. Since the focus of the chapter is assessing use of LIS for monitoring antimicrobial resistance, the information will focus on blood culture data from microbiology laboratories. As described above, DISALab LIS operates on Open DataBase Connectivity (ODBC) system, an open standard 'application programming interface' (API) for accessing databases.

4.1.6 Data flow from CDW to utilisation

Blood culture data from laboratories, are aggregated at the CDW. Here data can be extracted manually as requested by researchers for a particular purpose once an approval is granted by the data manager in-charge of the CDW. The CDW is directly interconnected with the DISALab, which means that the CDW personnel have direct access to data coming from all NHLS laboratories using the DISALab System.

To simplify the process, data are extracted manually with the aid of a well developed structured query language (SQL) program as shown in Table 2.1, Section 2.5 'data management'. The CDW composition is limited in terms of what epidemiological investigations could be executed from the data, as the blood culture data does not contain clinical parameters, hence investigations linking clinical outcomes to antimicrobial resistance may not be undertaken.

4.1.7 Critical assessment of challenges of LIS and data quality

The focus of this section is to understand the dynamics of the NHLS LIS, key operational challenges and its role in monitoring antimicrobial resistance to nosocomial pathogens in the country. The DISLab was designed to be flexible, changing and revolving all the time, hence it is subject to constant improvement.

4.1.7.1 Different version of DISALab

The NHLS LIS was running on different versions of DISALab due to differences in the roll out time and user preferences, such that changes that are suggested globally might not all be affected by different laboratory managers in different laboratories. Some laboratories might prefer to make few modifications based on their needs while others might not have changed anything at all. This made it difficult for all laboratories to work on standardised data protocols. Such a situation could have propagated differences in blood culture data quality, including differential AMR rates observed from various laboratories and which aggregates at the CDW. It might also be due to differential laboratory practices leading to selective testing of certain antibiotics. (section4.2, Table 4.1)

4.1.7.2 Replication and data errors

The CDW cannot replicate data in the database since data found at the repository were electronically transferred from the different sources. However, the CDW programmer does interrogate the data that gets extracted and performs data extraction error identification exercise as standard practice. The errors assessed are mostly those on codes that were used by the laboratories, e.g., the identification code in the CDW database for example Universitas hospital is 53. Before any changes could be effected on the data, ample verification of data is

done. Therefore, it is recommended to incorporate an error rate into the system that is universally acceptable.

4.1.7.3 Lack of access to original data source

The CDW does not have access to the original data source, such as the laboratory request forms. However, DISALab maintains master data (standard reference data) for all NHLS laboratories, which means that laboratories use the same table of codes. The master data is administered at the NHLS central corporate office and facilitates easier merging of data from different laboratories without any major problems.

4.1.7.4 Duplicate data

Duplicate entries can pose a challenge. The blood culture data entry must have the same ID number, name and surname, date collected, area/place, hospital and results. Unless the blood culture data entries have all similar records, that particular entry could only be assumed as a duplicate entry.

4.1.7.5 Variation in LIS

There are major challenges relating to the NHLS LIS, which might originate from wide variation in the operations of the LIS between different laboratories. These appear to lead to wide variations in procedures that are used for gathering and reporting of blood culture data. Some of the underlying causes of such wide variability might be:

- Different reporting styles between different laboratories, including different names used by different instruments.

- Instruments used vary between different laboratories (other laboratories use more advanced instruments than others) as documented in section 4.2

- Lack of standardisation across different laboratories which might affect scope of generated data.

4.1.7.6 Structure of LIS

Often unforeseeable errors might have been difficult to deal with despite the fact that the program has been regularly monitored to enhance accuracy of the data that was entered onto the LIS database. The system generates a turnaround time of blood culture results i.e. the system has the ability to demonstrate time in and time out in terms of the sample processing and results outcome. However, this approach might have been problematic in conditions when the date structures were different i.e. dd-mm-yy or yy-dd-mm, but also where time was not entered into the database. The system might then have registered '00' for such data points indicating missing data on time. For this reason, laboratory data has to be treated with caution due to such omissions.

4.1.7.7 Capacity of the LIS

How large is the interconnectivity? Due to the large capacity of data handling, the LIS can be challenging in terms of its effectiveness. Should the LIS be too large, it might not function effectively. Hence utilisation of individual or separate servers by each laboratory as a way of making the system effective, becomes a major limitation in terms of national antimicrobial resistance surveillance programs, as the individual local servers are not interconnected to each other hence aggregating data becomes problematic. This means that each server transmits data separately into one central repository. Due to the efficiency of the network

services, or phone line, not all data might end being transmitted to the central repository. Some data get lost en-route the electronic transmission.

4.1.7.8 Database structure

The CDW operates on a relational database model. For a researcher to access data from the central repository there is need to design a query to extract the data of interest, and then assess what each query is giving back in terms of the data parameters that a researcher is interested in. To improve validity and reliability of data extracted from the CDW, there is need to create a micro strategy for the LIS, which would enhance the data that an individual wants to retrieve.

4.1.7.9 Data Security System

The LIS data is password protected and each of the local laboratories has an electronic gate keeper, to monitor and minimise data errors that could happen, but also to access the data for research use (https://labresults.nhls.ac.za). Since data comes from varying sources, it's usually unclean with numerous errors hence strict measures need to be taken before making appropriate use of the data

4.1.7.10 Turn around time

In a situation where time is not recorded, it is advisable to use laboratory time as a starting point so as to be able to calculate the turn-around time for the laboratory results. However, if time entered into the system is 'o' instead of leaving the cell blank, the system will read '0' as real zero time instead of missing data. In this situation data would obviously end up being skewed as it would cause 'o' inflated data.

4.1.7.11 LIS Performance

To achieve sustained data quality, the following procedures need to be followed:

- The laboratory clerks that register patients' details into the LIS need to undergo regular intensive refresher training as they do not have basic knowledge of laboratory sciences as such they might not understand all processes involved with blood culture data.

- There is need to settle on a standard data collection tool, modify the tool as necessary as is possible and when required, so that the laboratories settles on the real required data elements and in so doing, enhance aggregation of quality data into the database.

- There is need to filter out data errors so that data suits the needs of the user. To thoroughly ascertain data quality, it is advisable to exclude irrelevant data elements in the database.

- For the researcher to be able to access valuable information there is need to invest a lot of input into the laboratory information system because correct input data is essential to ensure accuracy and reliability of blood culture data.

- There is need for standardisation of laboratory procedures and skills building i.e. providing similar training to laboratory technicians/technologists, registry clerks etc., across sites, with the aim of generating same competencies which will ultimately improve overall data output.

- Incorporation of an automatic review program into the LIS to detect data errors and ensure accuracy of the data at the point of data entry into the database system.

4.1.8 Laboratory methods

Different laboratories have different volumes of blood cultures being processed and possibly used different methods of blood culturing and susceptibility testing. The assumption was that if laboratories used different methods, they may produce results showing different antimicrobial susceptibility patterns which may be due to variation in the laboratory methods used for blood culture procedures.

Based on the above assumption, a further investigation was done to determine; i) whether laboratories use different blood culture methods and procedures, ii) whether different methods for susceptibility testing use different MIC breakpoints, existence of equalities and non-equalities among different methods applied in various laboratories, some of which might influence differences in resistant pattern. Detailed findings are highlighted in section 4.2.

The operations of clinical microbiology laboratories and LIS interconnectivity in South Africa may not be comparable to that of the western world, where they use machines to the high end of the spectrum, where all process are automated i.e. such as computerized clinical decision support systems (CCDSS) in the USA/UK, which are information technology-based systems designed to improve clinical decision-making. (112)

4.1.9 Future dimensions of LIS-Trackcare

The NHLS has embarked on upgrading the LIS to make it function in real time. The DISA Lab LIS, which has been in use for several years by the NHLS, has now been replaced by TrakCare laboratory information system. The TrakCare system is being rolled out in all NHLS laboratories throughout the nine provinces of the country. (107) Since the process is being implemented in phases, the NHLS will experience an overlap in the LIS operations. However, the ultimate goal is that NHLS LIS migrates completely from DISALab to TrakCare LIS.

There are challenges inherent with introduction of new system into a program. However, it is envisaged that the operations of the laboratories and turnaround time for results would improve greatly with a system that works on real time. Also that the interconnectivity will be enhanced such that patients' results from any province can be accessed nationwide as opposed to the current system where laboratories operate independent of each other, hence should a patient move to another province, his/her laboratory data could not be accessed. The new TrakCare LIS has an in-built capacity to synchronise data across various sites as a means of improving efficiency in patient follow up and billing for laboratory tests.

To overcome data transfer delays, TrakCare LIS system is programmed to use redundant telephone lines. The system was programmed to be linked to a wider network area as well as to the central server at NHLS head offices in Johannesburg. Since information will be directed to the central server from which it could be extracted for analysis. Patient follow up would be easier should a patient move from one geographical area to the next.

With the new LIS, patients'previous blood culture results, that were done at another clinic or laboratory will be traced backwards and followed up effectively. The system permits validation of data in an easier way through web access. However with the new LIS, a different database structure is being used hence current data from the DISA Lab LIS might possibly not be transferable. The system might also not allow direct local access to the data such as extraction for analysis but could be visualised across other clinics or hospitals since its web access. Patient data will be available even though not everyone will be able to access the data due to a built in password protection program.

Since the system operates in real time, attending doctors might be limited to access data pertaining to their patients only. There has been constant development of the LIS program to be able to address any new needs of the user because there are indications that the new system might not have the ability to constantly update compared to DisaLab. At the same time the new LIS looks to be labour intensive. Such a scenario might create a problem to balance reliability and functionality of the TrakCare LIS.

In comparison, the new system, TrakCare LIS, is designed to provide a web-based access, such that results for individual patients could be accessed anywhere the patient would go for medical services within the public sector system. TrakCare LIS interface is configured using the same standard tests that the NHLS laboratories perform as well as the general working style. However, TrakCare LIS system is not as flexible to the local needs of the laboratories as the DISA Lab system has been.

TrakCare LIS system only allows for global changes, i.e. changes to entire network. This means that any changes made to the system by the user within the NHLS network will be universal and will affect all NHLS laboratories across the country. This means that the local

IT personnel are left with no opportunity to modify the system so as to suit their local needs. However, the major advantage is that the TrakCare LIS has been designed, validated and standardised with contributions and close involvement of information technology (IT) managers from various NHLS sites, hence the system has a high acceptability level by the end users as it is being rolled out.

4.1.10 Blood culture data quality

To improve data quality and minimise improper estimates of antimicrobial resistance that could lead to misinterpretation of the findings, consider excluding from the database data elements that are not compatible. When manual data extraction of blood culture data is performed at the CDW, an individual researcher needs to systematically exclude certain data elements that are inappropriate compared with the data parameters normally encountered in surveillance analysis.

There is need to monitor specific areas where problems with data quality could be identified and appropriate intervention undertaken to improve data quality. To achieve this, the following issues need to be considered:

- Whether a researcher would accept at face value what was extracted from the database using the designed query?
- Whether a researcher would be able to make any request for specific, logical, clear and unambiguous data elements in the query design? (For more details, refer to 2.5 data management, section 2.5.1, Table 2.1).

To improve the quality of data aggregated at the CDW, there is great need for various players from the different NHLS laboratories to team up so as to minimise major variability of

antimicrobial resistance patterns that might be originating to a large extent from data entry errors. In addition laboratories should always run regular validation exercises to make sure that data gathered is accurate and reliable.

To improve surveillance of antimicrobial resistance there is a need for the NHLS to introduce instruments that could be effectively utilized to generate reliable data elements to be used for various epidemiological investigations as well as aid clinical decision making regarding bacteraemia episode by clinicians in the clinical departments.

4.1.11 Conclusion

The LIS was not primarily designed as a research or surveillance tool. Its function has been to generate data that could be used for appropriate and accurate billing of all tests done in the lab. Data has also been used to understand the volume of tests done, the time it takes to get results back to the patient and use such data to plan service delivery of the NHLS. We believe such a system can be used as an effective surveillance tool to monitor development of antimicrobial resistance to nosocomial pathogens in our population, since the process of acquiring blood culture data is inherently ongoing. Therefore, understanding the shortfalls of the system and suggesting ways of improving the overall system performance is a step in the right direction, if there is a well established and functioning antimicrobial resistance surveillance program. Such a program would enhance our ability to contain the growing crisis of antimicrobial resistance that threaten our ability to treat patients effectively in South Africa.

4.2 Differences in Laboratory Methodology and their Impact on Antimicrobial Resistance Surveillance

4.2.1 Introduction

Retrospective analysis of data from the CDW revealed that there were significant variations in patterns of antimicrobial resistance by hospitals. (Chapter 5, Table 5.2, Chapter 6, Table 6.7 and 6.8) We therefore aimed to understand whether differences exist in laboratory methodology and assess whether such differences could have a significant impact on quality of antimicrobial resistance data and patterns of resistance as observed.

4.2.2 Methodology

4.2.2.1 Design and study setting

This qualitative observational study was conducted between June 2011 and January 2013. The NHLS clinical microbiology laboratories servicing public tertiary hospitals associated with academic institutions in three provinces were involved in the study (details of the sites have been provided in Chapter 2, section 2.2). The map of the Republic of South Africa below highlights the geographical location of the study sites (Figure 4.3) where a systematic observation of laboratory procedures and practices was undertaken mostly within 1-2 working days per each laboratory.

4.2.2.2 Data sources and collection procedure

The procedure for gathering information followed a systematic approach which was a combination of: i) an in-depth orientation of the activities of the laboratory in general with a more in-depth focus on the activities of specific microbiology department, ii) series of

informal discussions with individual members of staff who do blood cultures in the department, iii) observation of the activities taking place in the laboratory in general, but with a focus on the microbiology laboratory specifically blood culturing as well as, iv) a question and answer feedback with members of the microbiology department at each hospital. Staff members in the IT department were also involved and this helped to gather detailed information on the functionality of the laboratory as well as data flow from the front desk, where specimen registration takes place. A standard observation checklist (Appendix 12.4) was specifically developed and used for this purpose, to ascertain standardised data collection in all the study sites.

Map of the Republic of South Africa
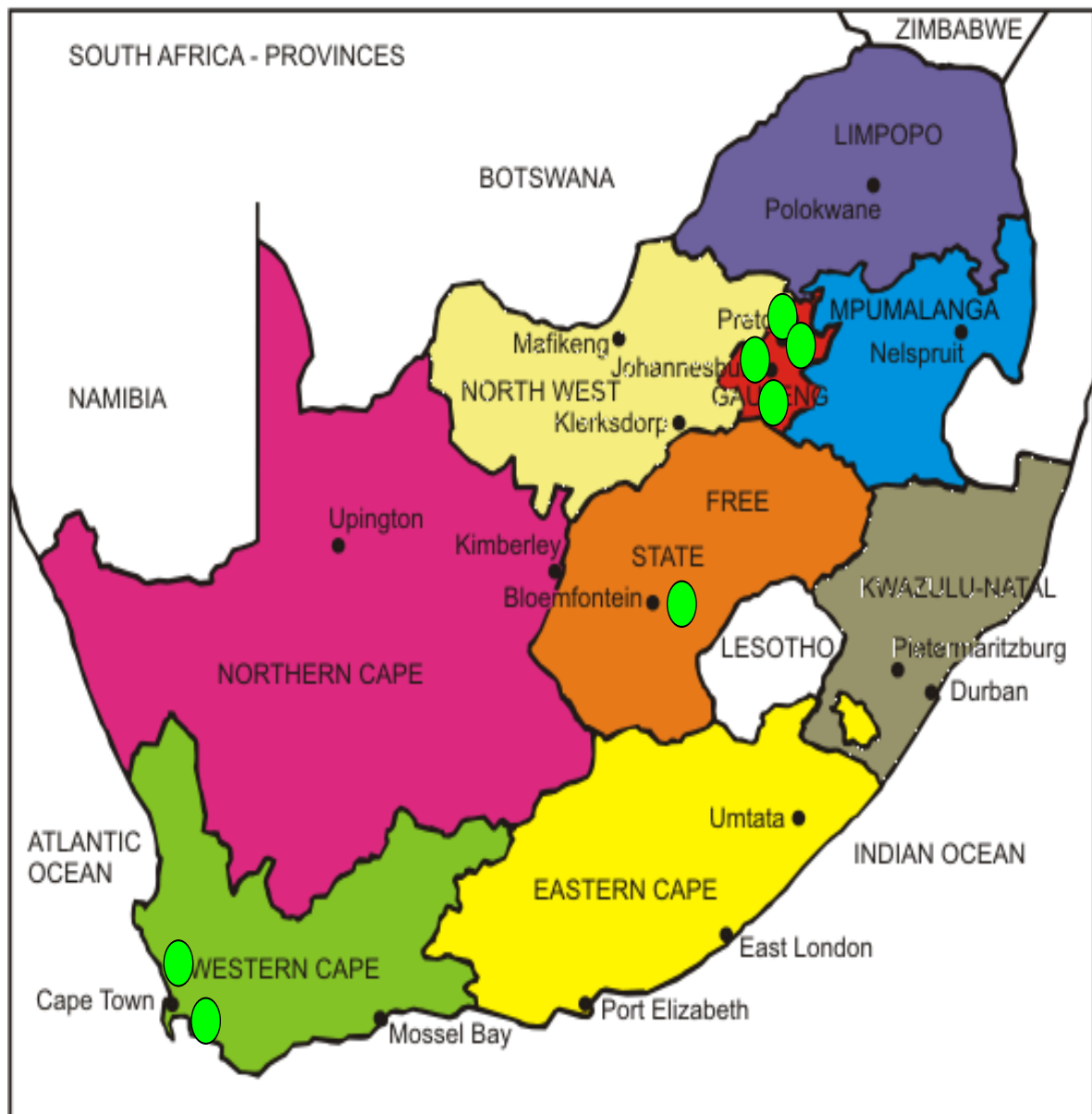
Figure 4.3 Map of the Republic of South Africa showing study sites

Free State province: Universitas Hospital complex; Western Cape Province: Groote Schuur & Tygerberg Hospital; Gauteng province: Charlotte Maxeke Johannesburg Academic Hospital, Helen Joseph, Chris Hani Baragwanath Hospital, Steve Biko Pretoria Academic Hospital.

4.2.2.3 Data sources and approach to collection

Interviews and informal discussions were done with laboratory staff in the microbiology department, who deal with specimens i.e. blood or cerebral spinal fluid (CSF), stool, urine, pass swabs, joint and peritoneal fluids for culturing. The purpose was to understand the operations of the laboratory regarding specimen registrations, processing, results dissemination, data entry as well as the interconnectivity of the LIS.

The pathologists provided more technical and academic details regarding blood culture procedures while laboratory managers provided more detailed information regarding the logistics and operations of the microbiology laboratory. In addition, laboratory technologists explained in detail the whole process of doing a blood culture and susceptibility testing (from specimen receipt to blood culture results, validation of results by pathologist and entry of results into LIS).

The registry clerks explained the details about specimen sorting from all clinical departments. The specimens are sent to the appropriate laboratory department; registration of the specimen to make sure that specimen identification is in line with patient identification. This undertaking by the laboratory staff eliminates the allocation of results to the wrong patient.

The IT managers gave a detailed description of the operations of the LIS, how data is transmitted to the central repository and limitations of the system, including data loss during electronic transmission from local server to the CDW.

4.2.3 Results

4.2.3.1 The results are summarised in the flow chart presented below

The chart below describes flow of blood culture specimens and results as well as the flow of data from the NHLS microbiology laboratories to the central repository (CDW).
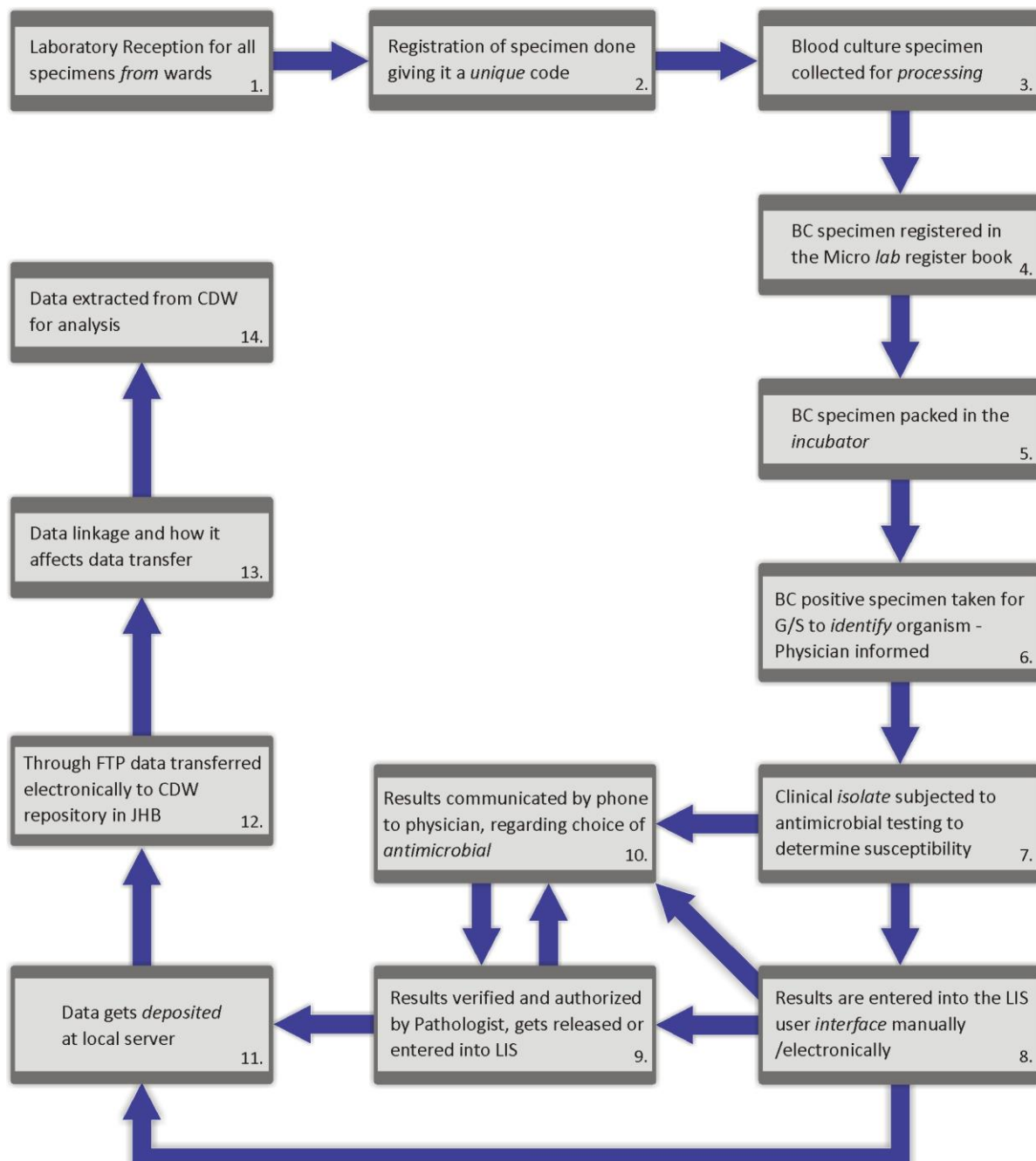
Figure 4.4 Diagrammatic representation of the NHLS blood culture data flow and interlinkage with the Laboratory Information System

Step 1: All specimens (blood culture and others) from the different clinical departments and wards are received in the laboratory central reception area.

Step 2: The specimens are registered and the following details are entered into the system: name of patient, ID number, ward where admitted and hospital, a unique code is allocated here. Patient details for each specimen are entered into the system and a code is generated for that particular patient. This code becomes the patient identifier and is pasted on specimen bottle for ease of identification. Such attention aids the laboratory staff to avoid giving results to the wrong patient.

Step 3: The specimens are then sorted in order of specimen type, i.e. blood culture, full blood count, urine, microscopy etc. Thereafter, specimens are distributed to the appropriate departments for processing.

Step 4: Blood culture are processed in the microbiology laboratory. Here, an active verification process of blood culture bottles is done against information documented on the laboratory request form from the clinical departments/wards. This process helps to ascertain that a particular specimen bottle belongs to a particular patient.

Step 5: Once the blood culture specimen bottle has been delivered to the microbiology laboratory, the specimens, first, get processed in the culture room. Each sample details are first entered into the register book thereafter each specimen bottle is inserted into the BacT/ALERT® 3D incubator, where blood culture specimen normally stay for up to a maximum of 5-7 days (Figure 4.5). This machine falls in the category of microbial detection system and is fully interfaced with the LIS program. Positive blood cultures are identified

each time an indicator light of the incubator bleeps up against a particular blood culture bottle. The positive culture bottle is then removed from the BacT/ALERT® 3D for further processing.

Figure 4.5 The pictograph of the BACT/ALERT 3D incubator.

This picture of the BA-3D was taken in the NHLS microbiology laboratory at the Charlotte Maxeke Johannesburg Academic Hospital. (P. Nyasulu)

Step 6: Gram staining is then done to identify the organism, and positive Gram stain results are then communicated to the physicians in the wards.

Step 7: Antimicrobial susceptibility testing is then carried out to find out which antibiotics the cultured bacteria are sensitive to. Usually within 2-28 hours after receipt of the blood culture specimen, the patient would have received the results at the Universitas hospital complex while at CMJAH blood culture turnaround time is ~48 hours from receipt of specimen to release of susceptibility results.

Step 8: Blood culture and antimicrobial susceptibility results are entered into LIS. If API method was used, the data are entered manually. The Vitek and Micro Scan (Figure 4.6) are fully automated machines. They are used for antimicrobial susceptibility detection. Results from the machine are automatically entered into the LIS since the interface of the microbiology detection machines feeds into the DISAlab. Antimicrobial susceptibility data from Vitek and Micro-Scan are electronically transmitted to the local server.

Figure 4.6 The pictograph of the Micro Scan

This picture of the MicroScan was taken in the NHLS microbiology laboratory at the Charlotte Maxeke Johannesburg Academic Hospital on the 5th of September, 2012. (P. Nyasulu)

Step 9: Once susceptibility results are ready and verified by the Pathologist, the clinicians are informed and guided accordingly on the choice of appropriate antibiotics to prescribe to patients. The Pathologist then signs off the printout of the results authorising the laboratory technician to enter results on the LIS computer and release the results to the clinical department or wards.

Step 10: Blood culture now on the LIS, can also be accessed directly by clinician in various clinical departments once an individual logs on to the DISALab LIS computer.

Step 11: From the automated machines, blood culture data automatically aggregates at the local server of each laboratory.

Step 12: Then from each local server, blood culture data are transmitted to the central data warehouse (CDW) in Johannesburg via the File Transfer Protocol (FTP). DISA Lab does not operate in real time hence regular transfer of data takes place.

Step 13: The major challenge encountered at steps 11 and 12, which can compromise data integrity is lack of appropriate data linkage, because the current set up can sometimes affect data transfer in times when the network or phone line is off or the LIS computer has a technical problem (Figure 4.7). In addition, time is an essential data component hence time gets recorded so that all changes made to the data at any point in time could be traced back, by looking at what time a particular individual made any change (this is a built-in security control method of DISA Lab).
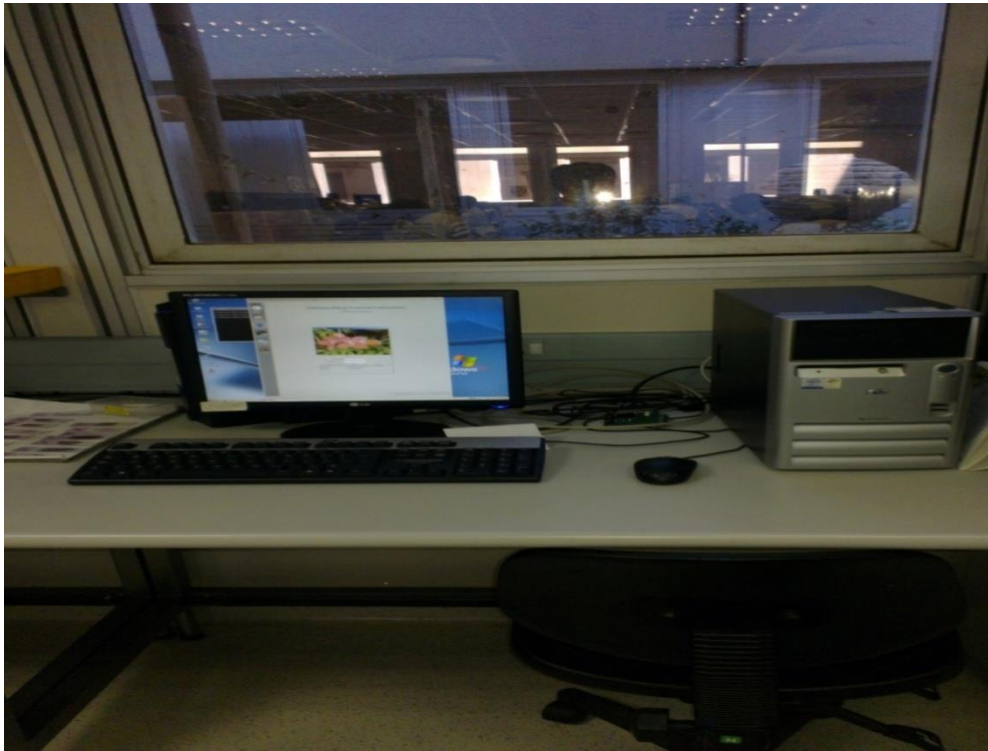
Figure 4.7 The pictograph of the LIS computer

This picture of the LIS computer is linked to all blood culture processing machines. It was taken in the NHLS microbiology laboratory at the Charlotte Maxeke Johannesburg Academic Hospital on the 5th of September, 2012. (P. Nyasulu)

Step 14: At the CDW, data extraction for analysis of antimicrobial resistance patterns from blood cultures takes place (Further details are given in section 4.1.6).

4.2.3.2 Standard operating procedures

Most microbiology laboratories operated on set standard operating procedures (SOPs). These procedures were available and visibly displayed in some but not all microbiology laboratories. For example, at the Universitas hospital complex, the SOPs were not seen, either they were not available or were just not displayed. This could be a sign that even if the SOP existed, it is not a common point of reference. However, in other microbiology laboratories such as Groote Schuur hospital, SOPs were visibly displayed and actively used as a point of reference. The following SOPs were noted at this site:

- MIC0712: Maintenance and loading the Bactec 9120/9240 blood culture system.

- MIC0713: Processing of Bactec culture bottles

- MIC 0732: Antimicrobial sensitivity testing

The microbiology laboratory at GSH follows the designed protocol on how antimicrobial susceptibility testing should be done, standard method (disk diffusion according to Kirby-Bauer, E-Test derivative of agar dilution etc.). Automated reading of the antimicrobial susceptibility test is done if performed through the computerised machines linked with the LIS. For other hospitals, similar standard procedures are followed with differences in their operations.

4.2.3.3 Similarities and differences in Laboratory Methods and Procedures

Table 1 summarises some of the observed similarities and differences in laboratory methods as well as equipments used for blood culturing in various NHLS laboratories.

Table 4.1 The table shows the comparative assessment of NHLS blood culture methodology

	Hospital				
Parameters Assessed	*CMJAH*	*CHB*	*SBPAH*	*HJ*	*TH*
Micro-SOP	√	√	√	√	√
Automated organism identification/susceptibility testing	Microscan	Microscan	Vitek 2	Microscan	Vitek 2
Automated Blood Culture	BA-3D	BA-3D	BA-3D	BA-3D	BA-3D
Specimen registration	√	√	√	√	
Results entry	Manual	Manual	Manual	Manual	Manual
LIS access in the ward	√	√	√	√	√
Results validation	Registrar	Pathologist	Pathologist	Pathologist	Pathologist
CPU	√	√	-	√	-
Susceptibility testing guidelines	CLSI	CLSI	CLSI	CLSI	CLSI

From table 4.1, we concluded that no major differences exist in the methods of blood culturing and susceptibility testing between different NHLS sites. The microscan and vitek2 systems were validated and pathologists were of the opinion that these machines produced similar results.

4.2.3.4 Limitations

There were over 300 NHLS laboratories nationwide during the and over 50 of these were in KwaZulu Natal (KZN). This means that only about 250 of these NHLS laboratories operated on a FTP system transferring data from the microbiology laboratories to the CDW. In this case, data functionality might be compromised due to other network program activities of the system.

4.2.4 Discussion and Conclusion

We observed that different laboratories use different volumes of blood sample in processing blood cultures and subtle differences in methods of blood culturing and susceptibility testing. The study was done to address the assumption that if different methods were used, then the laboratories may produce different antimicrobial susceptibility culture results simply because the methods used are different and not because the differences are geographically inherent.

There were few observed differences in the operational procedures as well as the microbial detection systems used in different laboratories. Such differences might not influence blood culturing outcomes that could lead to major differences in resistance pattern. In addition, some laboratories such as GSH, are better equipped than others such as UH, which might be an indication of differences in the distribution and or availability of resources. There is need

to understand equalities and non equalities among the different laboratory methods as some of these might influence differences in resistant pattern.

In addition, since data is not in real time and that the system operates on multiple servers transferring data from local servers into one central repository, there is need to design a program that could be executed to extract data for analysis and reporting each time data are required.

Chapter 5   Evaluating the suitability of the LIS as a monitoring tool for recording antimicrobial resistance trends and patterns in tertiary public hospitals in South Africa

This chapter provides findings of a critical assessment of LIS through analysis of blood culture data from seven NHLS clinical microbiology laboratories aggregated at the CDW from 2005 to 2009. The aim was to assess reliability of the NHLS LIS as a tool for reporting antimicrobial resistance among blood culture isolates of nosocomial bacterial pathogens from public hospitals in South Africa.

5.0 Abstract

Aim: To evaluate suitability of a laboratory information system (LIS) on reporting prevalence, patterns and time trends, and associated demographic factors of resistance to commonly used antibiotics for selected pathogens from blood specimens.

Methods: A retrospective analysis was conducted of routine data recorded on the LIS of blood-culture isolates of *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), and *Pseudomonas aeruginosa* (PA) collected by the National Health Laboratory Service (NHLS) between July 1, 2005 and December 31, 2009 from diagnostic microbiology laboratories at 7 tertiary public hospitals in South Africa. Antimicrobial resistance to commonly used antimicrobials was systematically recorded and analysed. Multivariate logistic regression models were used to assess factors associated with antimicrobial resistance.

Results: Information on 9969 isolates was available, of which 3942 (39.5%), 4466 (44.8%) and 1561 (15.7%) were SA, KP and PA, respectively. The proportion of resistance across all antibiotics tested was highest in the 30-39 year age-group for SA (28.4%) and PA (51.5%), but for KP, the highest proportion (73.3%) was in the 5-9 year age-group. SA and PA resistance was similar between males and females. For KP, a higher percentage of the isolates from females were resistant. The highest proportion of resistance at specific sites to non-wild type isolates was as follows: 47.9% of SA resistant isolates were from Tygerberg hospital, 72% of KP resistant isolates were from Universitas hospital and 67.1% of PA resistant isolates were from Steve Biko Pretoria Academic hospital. SA resistance to cloxacillin was 39% and to vancomycin <0.1%. KP resistance to carbapenems was low; imipenem 0.1% (range 0%-0.5%) and meropenem 0.1% (range 0%-0.3%), ertapenem 2% (range 0.5%-4.6%) - as was resistance to colistin 1.7% (range 0-2.6%). PA resistance to colistin was 1.9% (range 0.0 -13.3%). There was a significantly increasing trend of KP resistance to ciprofloxacin (32.6% to 64.9%, p<0.001), cotrimoxazole (67.5% to 81.6%, p<0.001) and cefotaxime-ceftriaxone (55.5% to 73.2%, p<0.001) over the study period. PA resistance to meropenem showed a significant increasing trend from 2006 (27.5%) to 2009 (53.9%) (p<0.001). Age group <5 years, female gender, hospital location and year of infection were significantly associated with higher antimicrobial resistance.

Conclusions: The proportion of antimicrobial resistance reported by the LIS was high and shows a significant increasing trend among individual agents, i.e. ciprofloxacin, cotrimoxazole among others. Enhancement of continued surveillance of antimicrobial resistance among bloodstream hospital-acquired infections is recommended. Such data would aid the understanding of the magnitude of the problem and provide solid evidence upon which policies and practices aimed at containing antimicrobial resistance could be generated.

5.1 Introduction

The magnitude of antimicrobial drug resistance has accentuated the need for continued surveillance of antimicrobial susceptibility.(76, 113, 114) Resistance of bacterial pathogens to conventional antimicrobials has become a global problem with hospital infections becoming more challenging among immune-compromised individuals, emphasizing the importance to systematically monitor patterns and trends of antimicrobial resistance over time. (16, 115, 116)

Enhanced information retrieval and better understanding of the magnitude of the problem would facilitate timely implementation of appropriate interventions including review of antimicrobial prescriptions policy and treatment guidelines that would reinforce prudent antimicrobial use. (117-119) In the face of a decline in the development of new antimicrobial drugs by pharmaceutical companies, the long-term goal of patient management would be to preserve the effectiveness of currently available antimicrobials so that they would remain functional for years to come. (16)

Surveillance networks such as the European Antimicrobial Resistance Surveillance System - EARSS (Europe) (120) and the National Nosocomial Infections Surveillance System-NNIS (USA) (121) have been established over the years focusing on pathogens that serve to provide reliable sources of antimicrobial susceptibility data. Such data have been used to determine resistance patterns and monitor emerging antimicrobial resistance. (17) However, at present there is scarcity of data from most developing countries regarding the burden of antimicrobial resistance, even among nosocomial pathogens which reflect the situation in hospitals from where most resistance problems have emerged. A recent systematic review showed evidence of resistance to commonly used antimicrobial drugs in the South African population. The

proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) was 35% while *Klebsiella pneumoniae* showed increasing resistance to 3rd generation cephalosporins or isolates producing extended-spectrum beta-lactamases (ESBLs) from 33% to 49%, and from 18% to 28% for fluoroquinolones in academic hospitals from 1999 and 2007. Resistance among *Pseudomonas aeruginosa* isolates to ciprofloxacin was 43%. (31) Antimicrobial resistance is a major catalyst for therapeutic failure of antimicrobial agents prescribed empirically. Frequently in low resource settings where laboratory facilities are not available, clinicians have to rely on clinical diagnosis and empirical treatment for patient management. (75, 113, 122)

Knowledge of local prevalence of pathogens and antimicrobial resistance serves as a guide for routine antimicrobial prescription. Ideally, clinical decision-making regarding choice of effective antibacterials should be guided by global (for empiric treatment) and local knowledge of antimicrobial resistance epidemiology. (122) In addition, antimicrobial resistance surveillance data would guide planning of targeted public health interventions to control the development of antimicrobial resistance and spread of resistant pathogens in hospitals. (16)

Data on resistance patterns would augment infection control measures and promote improved antimicrobial prescribing habits among clinicians. (115) This study investigated the suitability of Laboratory Information System (LIS) reporting of antimicrobial resistance prevalence, patterns and temporal trends as well as demographic factors associated with antimicrobial resistance among three selected pathogens, *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA), causing blood stream infections in patients admitted at tertiary public hospitals in South Africa.

5.2 Methodology

5.2.1 Study Design

This was a retrospective analysis of routine blood culture data reported from 2005-2009 by the NHLS and extracted from the CDW situated at the corporate office of the NHLS at Sandringham, South Africa. The study was approved by the Human Research Ethics Committee, University of the Witwatersrand, approval number M10625 (Appendix 12.3.8).

5.2.2 Participating Institutions

Seven tertiary public hospitals were included in the study: Charlotte Maxeke Johannesburg Academic hospital (CMJAH), Steve Biko Pretoria Academic hospital (SBPAH), Chris Hani Baragwanath hospital (CHBH) and Helen Joseph (HJ) from Gauteng province; Universitas Hospital (UH) from Free State province; Groote Schuur (GSH) and Tygerberg hospitals (TH) from the Western Cape. All the hospitals involved were associated with academic institutions.

5.2.3 Laboratory Methods

The NHLS academic laboratories used the automated BactAlert system for blood culture investigations and automated MicroScan or Vitek 2 systems, or conventional biochemical methods for identification of pathogens. Antibiotic susceptibility testing was done following the Clinical Laboratory Standards Institute (CLSI) guidelines. Various methods were used including testing by disk diffusion technology such as the Kirby-Bauer and Etest methods or automated testing using MicroScan or Vitek 2 systems. Quality control for susceptibility

testing was taken into account at each participating site. It is standard practice worldwide for quality control procedures to be used for drug susceptibility testing, including the use of dedicated international strains such as *S. aureus* for Gram-positive bacteria and *E. coli* for Gram-negatives, as well as standardization of inoculums size and incubation period. Only single episodes of bacteraemia were recorded by the laboratory to avoid bias in susceptibility reporting. Table 5.1 below shows details of methods of susceptibility testing for blood culture isolates in use by participating microbiology laboratories for the selected organisms. (123)

Table 5.1 Laboratory methods used in testing for antimicrobial susceptibility of Gram-negative bacilli and *Staphylococcus aureus*

Organism Group	CHB	CMHAH	SBPAH	HJ	UH	GSH	TH
Gram-negative bacilli	^MicroScan	MicroScan	Vitek 2*	Vitek 2	Disc diffusion	Vitek 2	Vitek 2
Staphylococcus aureus	Disc diffusion, Etest	Disc diffusion, Etest MicroScan	Vitek 2* Etest	Disc diffusion Etest	Disc diffusion	Disc diffusion, Etest	Disc diffusion Etest

*Vitek: BioMerieux, North Carolina. ^MicroScan: Dade Behring Inc, Califonia

5.2.4 Data Extraction

Data were extracted on all blood culture positive isolates of SA, KP and PA reported within the study period by DISA-LIS at NHLS. Susceptibility data reported by DISA were extracted from the CDW data repository by running SQL query from several database servers, details are described in chapter 2 section 2.5.1. Blood culture data of isolates from all wards including casualty department reported between July 1, 2005 and December 31, 2009 were included. All demographic and microbiological variables to be included in the analysis were extracted. Patterns and trends of resistance were expressed in terms of the number of non-wild type isolates divided by the total number of blood culture isolates for each organism.

The variable 'resistance' was inferred when the growth of an isolate was found to be inhibited at internationally recognised "critical concentrations" of the antibiotics on susceptibility testing. Other covariates were demographic and geographic characteristics including: age, gender, province and names of hospitals, wards and year of data collection.

5.2.5 Statistical Analysis

Data were checked and cleaning for each included variable was done. Data were then analysed using Stata version 11 (StataCorp Limited, College Station, Texas, USA). Univariate analysis was done to describe the frequency distribution of the selected pathogens as well as the distribution of the proportion of resistant isolates of the selected pathogens per antibiotic tested. Associations between resistance and various presumed risk factors (province, organism, age, gender, hospital wards and specimen collection year) were analysed using Pearson chi-square test for categorized variables. A multivariate logistic regression model was used to investigate independent predictors of antibiotics-specific resistance as well as composite resistance based on a set of antibiotics. Two-sided p values of <0.05 were considered significant.

5.3 Results

5.3.1 Demographic and geographical characteristics of bacteraemia episodes

There were 9969 single bacteraemia episode-linked isolates of selected pathogens within the study period of which 3942 (39.5%) were SA, 4466 (44.8%) were KP and 1561 (15.7%) were PA. The <5 years age-group had the most blood stream isolates in the case of each of the three respective pathogens: mean of 306 SA episodes per annum for the first 4 years of life, 418 episodes of KP and 111 of PA. Comparable mean annual figures for the 20-59 years age-

group were 48.5 episodes of SA, 48.2 of KP and 19.9 of PA, i.e. 6.3 times fewer cases of SA, 8.7 times fewer of KP and 5.6 times fewer of PA episodes than in the <5 years age-group.

There were more bacteraemic episodes caused by each of the three pathogens in males than in females. The proportion of bacteraemic episodes in relation to numbers of admissions and duration of patients' stay in hospital is not available for comparison of frequency of organism-specific bacteraemic episodes between hospitals. However, considering the relative percentages of organism-specific episodes in each hospital, SA episodes at Helen Joseph (49.8%), Tygerberg (47.1%) and Groote Schuur (45.5%) against mean of 40.3% of all 7 hospitals; KP episodes at Universitas (52.1%) and SBPAH (51.3%) against the 7-hospital mean of 45.5%; and PA episodes at SBPAH (20.1%) and CMJAH (19.2%) against the 7-hospital mean of 15.2% suggest possible excess of SA, KP and PA cases at the named hospitals. Because of confounding factors, the validity of such an approach is questionable. The numbers of episodes per annum for the respective pathogens for the period 2006-2009, varied from 871 to 965 (mean 902) for SA, 974 to 1124 (mean 1030.5) for KP and 347 – 368 (mean 357) for PA. (Table 5.2)

Table 5.2 Distribution of demographic and geographical characteristics of patients presenting with bacteraemia episode caused by the three organisms

Characteristic	*Staphylococcus aureus*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*
Age	n/N *(%)	n/N *(%)	n/N *(%)
<5	1224 (31.1)	1673 (37.5)	444 (28.4)
5-9	95 (2.4)	60 (1.3)	34 (2.2)
10-19	231 (5.9)	194 (4.3)	67 (4.3)
20-29	504 (12.8)	439 (9.8)	223 (14.3)
30-39	598 (15.2)	611 (13.7)	235 (15.1)
40-49	482 (12.2)	458 (10.3)	188 (12.0)
50-59	354 (9.0)	421 (9.4)	151 (9.7)
60-69	270 (6.9)	336 (7.5)	126 (8.1)
≥70	184 (4.7)	274 (6.1)	93 (6.0)
Gender			
Male	2185 (57.4)	2421 (56.0)	858 (57.0)
Female	1619 (42.6)	1902 (44.0)	648 (43.0)
Hospital			
Charlotte Maxe JAH	611 (15.5)	670 (15.0)	304 (19.5)
Chris Hani Bara	1120 (28.4)	1382 (30.9)	454 (29.1)
Helen Joseph	374 (9.5)	268 (6.0)	109 (7.0)
Steve Biko PAH	438 (11.1)	786 (17.6)	307 (19.7)
Universitas	173 (4.4)	261 (5.8)	67 (4.3)
Groote Schuur	556 (14.1)	531 (11.9)	135 (8.7)
Tygerberg	670 (17.0)	568 (12.7)	185 (11.9)
Province			
Gauteng	2543 (64.5)	3106 (69.6)	1174 (75.2)
Free State	173 (4.4)	261 (5.8)	67 (4.3)
Western Cape	1226 (31.1)	1099 (24.6)	320 (20.5)
Year			
2005	335 (8.5)	344 (7.7)	133 (8.5)
2006	965 (24.5)	974 (21.8)	355 (22.7)
2007	849 (21.5)	1002 (22.4)	358 (22.9)
2008	922 (23.4)	1124 (25.2)	347 (22.2)
2009	871 (22.1)	1022 (22.9)	368 (23.6)

*The proportions (%) are number of isolates for each characteristic (n) / total number of isolates for each individual pathogen (N). The total number of isolates for each characteristic were SA =3 942; KP = 4466; PA = 1561, except for gender, where the total number of isolates were: SA = 3804; KP = 4323; PA = 1506 due to missing data on gender.

5.3.2 Distribution of antimicrobial resistance rates among selected pathogens

The pattern of resistance to various antibiotics was fairly similar between the three pathogens.

For S. aureus, resistance is still detected for linezolid (0 out of 70) and minimal (1 out of 865) for vancomycin and is <15% for fusidic acid. For other antibiotics, SA resistance is above 20% with variation between individual antibiotics and >15% across all ages for clindamycin. For KP, resistance is almost not detected for meropenem and imipenem and slowly gaining ground for etrapenem. Resistance to amikacin is below 30% while for the rest of the antibiotic resistance is >30% across all age-groups. Among PA isolates resistance rate was mostly >30% across all age categories for all antibiotics except for ceftazidime that showed resistance rate of below 20%. Figure 5.1* - 5.3 below shows the distribution of antimicrobial resistance rate of SA, KP and PA by Age-group.
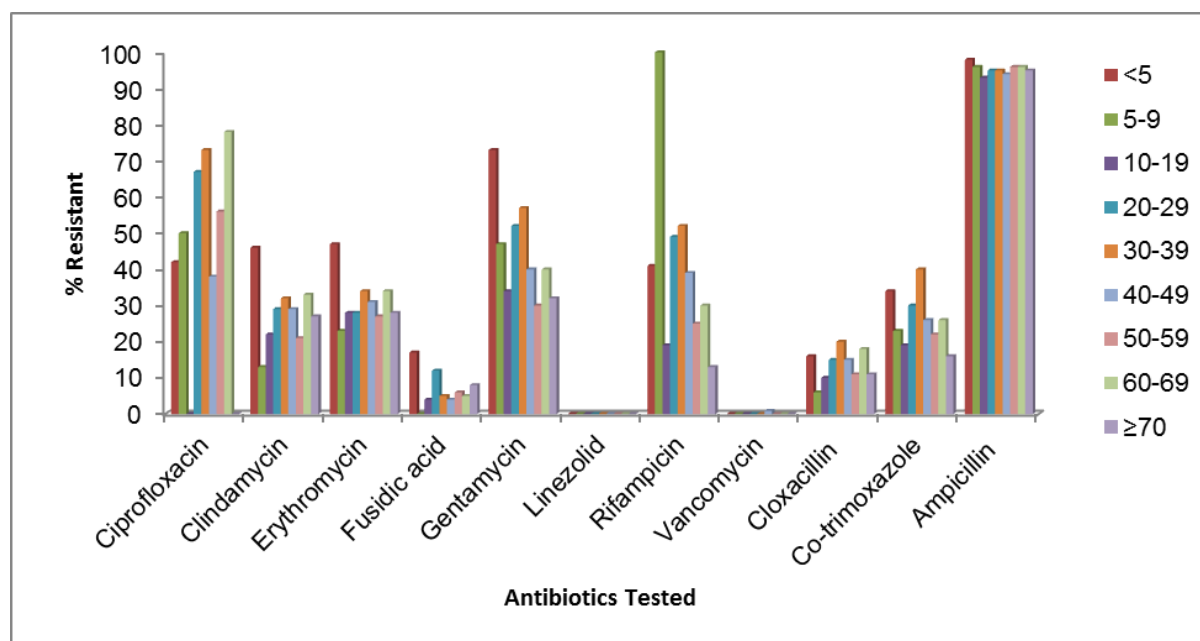
Figure 5.1 Antimicrobial resistance rate of *Staphylococcus aureus* isolates for the period 2005 to 2009 by age-group

*There were only 3 isolates investigated for rifampicin resistance and all three were resistant. The percentage figures for rifampicin resistance in the various age groups should therefore be treated with reserve.
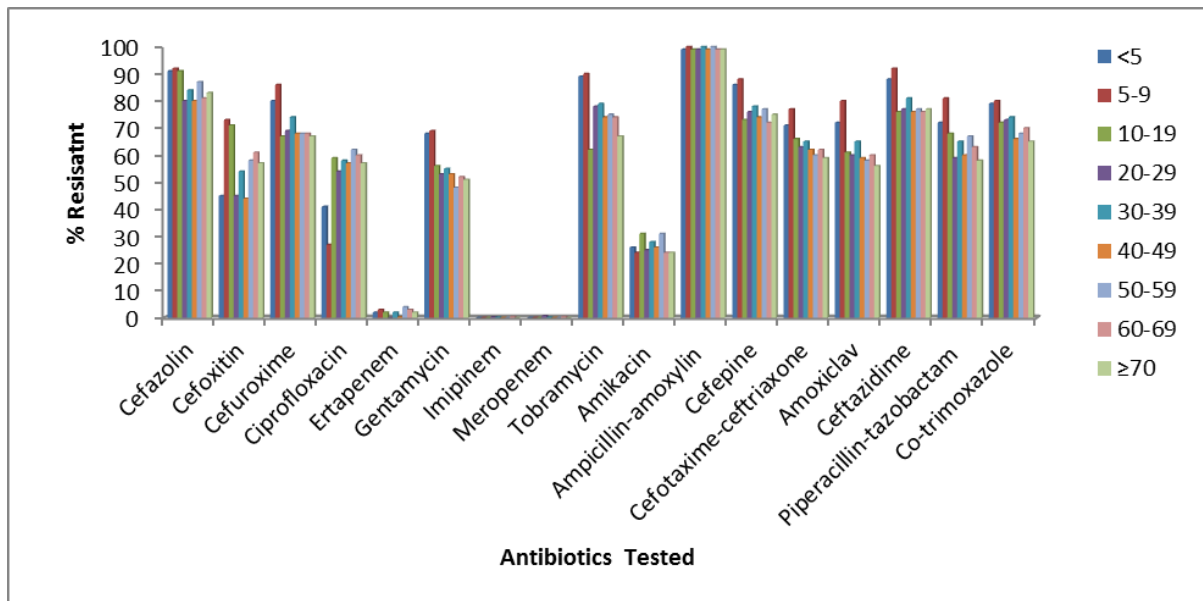
Figure 5.2 Antimicrobial resistance rate of *Klebsiella pneumoniae* isolates for the period 2005 to 2009 by age-group
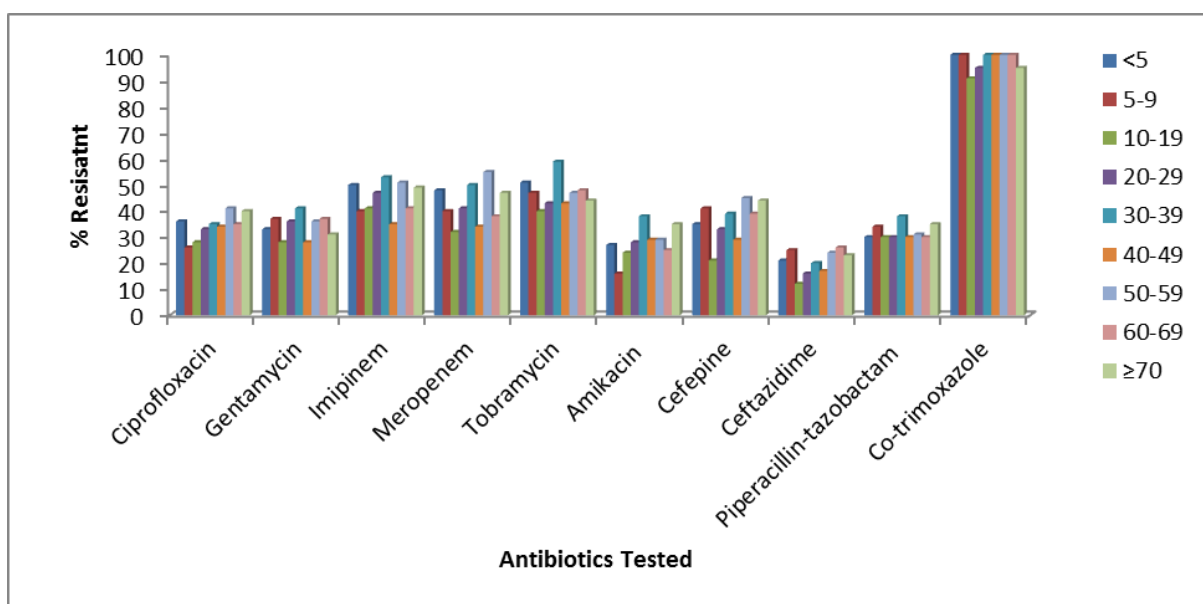
Figure 5.3 Antimicrobial resistance rate of *Pseudomonas aeruginosa* isolates for the period 2005 to 2009 by age-group

5.3.3 Distribution of antimicrobial resistance rate by gender

There is a preponderance for higher proportions of resistant isolates, >60% of SA resistance isolates to various antibiotics among males compared to females for ciprofloxacin, fusidic

acid, gentamicin and ampicillin and lower proportions, <40% of SA resistant isolates among males than females for clindamycin, erythromycin and cotrimoxazole. SA resistance to rifampicin and cloxacillin appears similar. However, the observed variation in proportions of resistant isolates between males and females was not significant. The trend of KP resistance shows that proportion of resistance is lower among males compared to females among most antibiotics with the exception of amikacin, the rate is higher among males compared to females, but has similar proportions of resistance between males and females for ciprofloxacin and ampicillin.

For PA, the proportions of resistance between males and females is almost similar for most of the antibiotics except for ciprofloxacin, gentamicin and imipenem, where the resistance is >30%, while for meropenem and tobramycin, the proportion of resistance is higher among females than males with resistance >40%. No real difference in proportion of resistance was observed for other antibiotics, i.e. cefepime and ceftazidime. Figure 5.4-5.6 below shows the distribution of antimicrobial resistance rate of SA, KP and PA by gender.
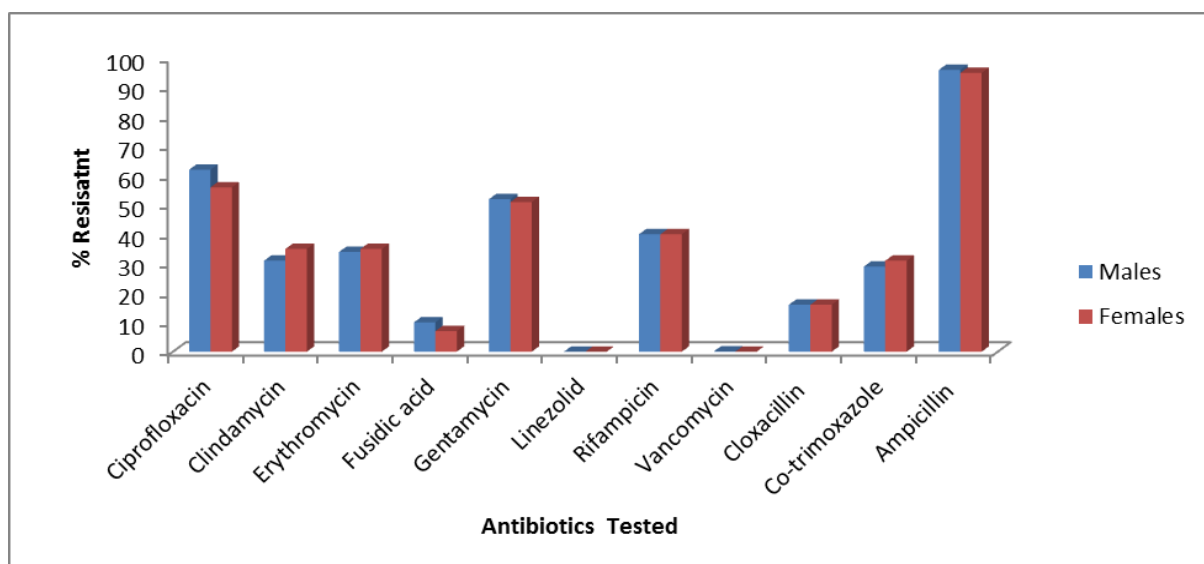
Figure 5.4 Antimicrobial resistance rate of *Staphylococcus aureus* isolates for the period 2005 to 2009 by gender
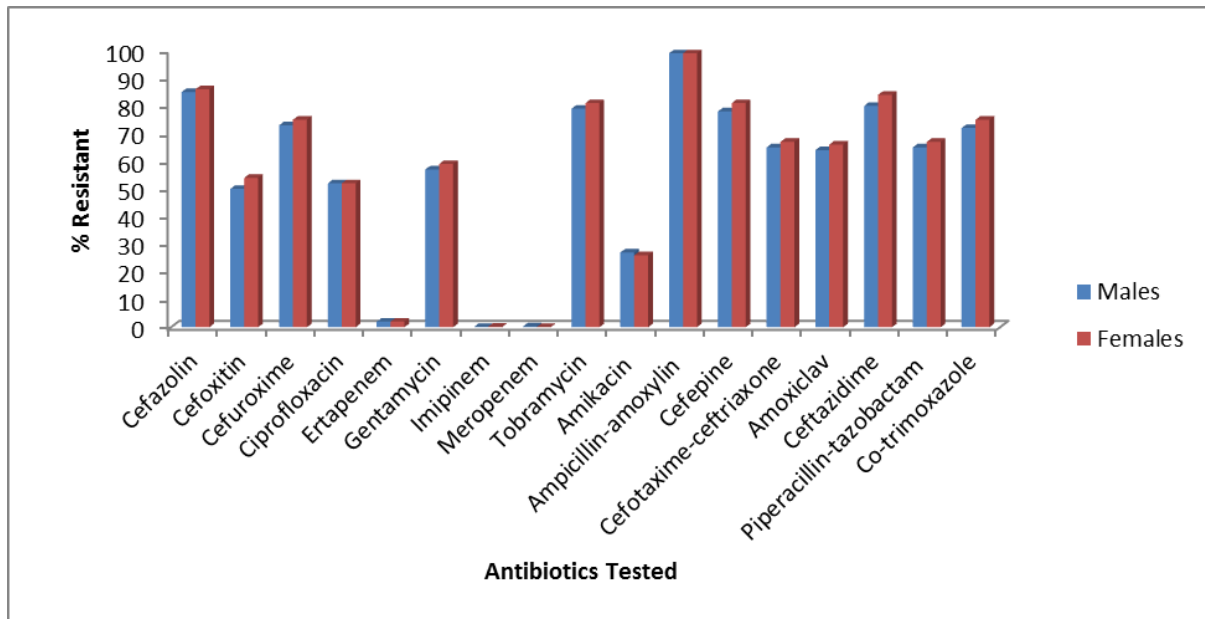
Figure 5.5 Antimicrobial resistance rate of *Klebsiella pneumoniae* isolates for the period 2005 to 2009 by gender
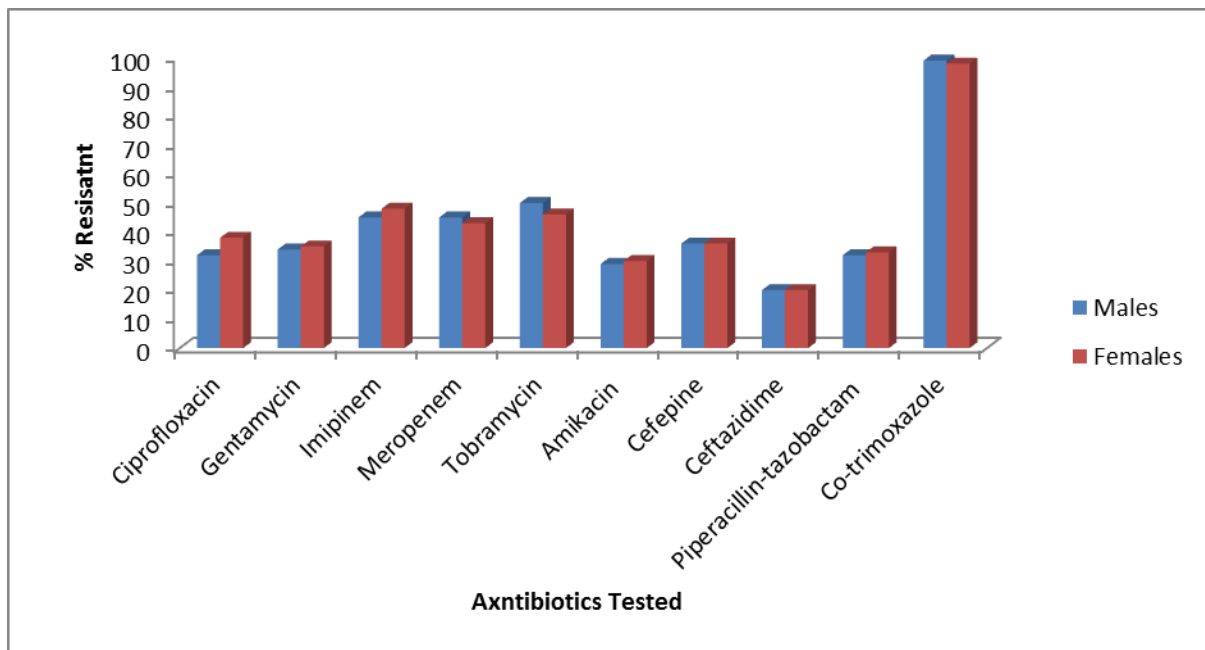
Figure 5.6 Antimicrobial resistance rate of *Pseudomonas aeruginosa* isolates for the period 2005 to 2009 by gender

5.3.4 Patterns of *S. aureus* resistance

SA resistance to ciprofloxacin ranged 20% - 100% across all sites. There was no data for ciprofloxacin susceptibility testing at SBPAH and scanty data at CHB, HJ, UH and TH. The Frequencies of clindamycin (72%) and rifampicin (60.7%) resistance in SA at TH are high and do not appear to be linked to macrolide (erythromycin) resistance (44.1%). Resistance to vancomycin was <0.5% and the rate of MRSA were highest at TH 43% (range 0.4% - 43%, there is a concern about recording resistance to cloxacillin or cefoxitin in LIS at different sites). Resistance to cotrimoxazole ranged 25% - 37% and gentamicin resistance ranged from 40% - 67% across all hospitals. There was scanty data for other drugs to make any meaningful analysis. (Table 5.3)

Table 5.3 Antimicrobial resistance patterns of selected blood borne infections, by hospital, during 2005 – 2009 period

Organism/drug	Total	CMJAH	CHB	HJ	SBPAH	UH	GSH	TH	p-value
Staphylococcus aureus	% (n/N)**								
Ampicillin	95.7(3,322/3,471)	96.8(481/497)	98.8(941/952)	97.4(295/303)	88.2(365/414)	92.1(140/152)	94.7(482/509)	96.0(619/644)	0.85
Cloxacillin	15.4(588/3,828)*	4.2(25/595)*	0.4(4/1,103)*	3.8(14/366)*	5.0(21/422)	30.4(51/168)	37.0(193/522)	43.0(280/652)	<0.001
Vancomycin	0.12(1/865)	0.0(0/34)	0.0(0/11)	0.0(0/25)	0.0 (0/37)	0.0 (0/108)	0.0 (0/337)	0.3(1/313)	0.94
Gentamicin	51.8(428/827)	40.0(6/15)	25.0 (3/12)	66.7(6/9)	22.2(2/9)	50.0(1/2)	46.6(132/283)	55.9(278/497)	0.02
Erythromycin	34.5(802/ 2,326)	40.5(104/257)	26.6(92/346)	53.6(59/110)	19.0(65/343)	40.9(54/132)	29.7(153/515)	44.1(275/623)	<0.001
Linezolid	0.0(0/70)	0.0(0/6)	0.0(0/4)	0.0(0/3)	0.0(0/1)	0.0(0/32)	0.0(0/15)	0.0(0/9)	na
Clindamycin	32.4(650/2,005)	17.9(40/224)	23.6(77/326)	32.1(27/84)	14.6(50/343)	33.1(47/142)	28.2(147/522)	72.0(262/364)	<0.001
Rifampicin	39.9(194/486)	25.0(3/12)	0.0(01/14)	55.6(5/9)	-	30.0(3/10)	32.1(95/296)	60.7(88/145)	<0.001
Fusidic acid	8.5(33/388)	11.1(1/9)	9.1(1/11)	0.0(0/3)	0.0(0/3)	25.0(2/8)	0.7(2/269)	31.8(27/85)	<0.001
Cotrimoxazole	29.9(577/1,930)	36.8(89/242)	30.9(100/324)	27.9(22/79)	50.0(3/6)	28.5(43/151)	24.6(128/521)	31.6(192/607)	0.02
Klebsiella pneumonia	% (n/N)**								
Ampicillin_Amoxy	99.5(3,769/ 3,789)	99.8(617/618)	99.5(1298/1305)	100.0(253/253)	99.3(739/744)	98.4(246/250)	99.3(277/279)	99.7(339/340)	0.16
Amoxiclav	64.8(2,550/3,936)	64.0(336/525)	73.0(869/1,190)	64.4(154/239)	59.2(439/741)	55.7(113/203)	52.7(263/499)	69.8(376/539)	<0.001
Imipinem	0.1(4/3,059)	0.0(0/386)	0.2(2/863)	0.0(0/156)	0.0(0/568)	0.0(0/221)	0.2(1/493)	0.3(1/372)	0.79
Meropenem	0.1(5/3,046)	0.0(0/397)	0.1(1/890)	0.0(0/162)	0.2(1/565)	0.0(0/141)	0.2(1/495)	0.5(2/396)	0.66
Ertapenem	2.0(50/2,474)	2.6(9/349)	2.8(18/652)	3.5(5/145)	0.8(4/533)	4.6(10/220)	0.8(3/376)	0.5(1/199)	0.01
Cefazolin	86.3(1,864/2,161)	94.8(349/368)	97.1(789/813)	90.9(140/154)	65.2(329/505)	77.8(168/216)	0.0(0/2)	86.4(89/103)	<0.001
Ceftazidime	82.0(2,428/2,962)	91.1(346/380)	95.5(799/837)	91.7(144/157)	69.1(425/615)	91.3(94/103)	58.7(289/492)	87.6331/378)	<0.001
Cefuroxime	73.8(2,437/3,301)	82.3(354/430)	93.3(746/800)	81.4(144/177)	62.6(423/676)	61.6(138/224)	59.5(297/499)	67.7(335/495)	<0.001
Cefoxitin	52.0(357/687)	65.6(40/61)	73.0(65/89)	42.9(21/49)	49.3(213/432)	37.5(3/8)	23.8(10/42)	83.3(5/6)	<0.001
Cefotaxime/ceftriaxone	66.0(2,238/3,390)	58.3(342/587)	65.1(822/1,263)	58.0(148/255)	68.1 (425/624)	92.5(124/134)	59.9(161/269)	83.7(216/258)	<0.001
Cefepime	79.8(2,364/2,963)	92.9(315/339)	94.4(794/841)	92.5(135/146)	69.8(396/567)	55.7(107/192)	59.5(292/491)	84.0(325/387)	<0.001
Piperacillin-tazobactam	66.3(1,820/2,745)	75.5(247/327)	83.9(590/703)	79.8(138/173)	69.7(430/617)	51.6(96/186)	29.6(137/463)	65.9(182/276)	<0.001
Gentamicin	58.7(2,242/3,820)	53.2(289/543)	69.5(726/1,045)	55.2(123/223)	53.2(387/723)	56.7(136/240)	57.8(289/500)	54.0(292/541)	<0.001
Tobramycin	80.4 (1,564/1,946)	89.6(233/260)	95.9(561/585)	89.6(103/115)	93.0(40/43)	60.0(102/170)	55.9(264/272)	86.7(261/301)	<0.001
Amikacin	26.4(695/ 2,631)	21.3(62/291)	33.8(162/479)	21.9(28/128)	40.2(471/674)	17.2(33/192)	14.9(74/498)	17.6(65/369)	<0.001
Ciplofloxacin	51.8(1,380/2,666)	48.5(191/394)	55.6(424/763)	77.7(139/179)	47.6(280/588)	43.1(56/130)	46.5(133/286)	48.2(157/326)	<0.001
Nalidixic-acid	83.8(586/699)	93.2(82/88)	95.4(313/328)	85.9(73/85)	70.2(33/47)	-	53.2(67/126)	72.0(18/25)	0.001
Nitrofurantoin	92.3(262/284)	75.0(6/8)	80.0(4/5)	95.5(63/66)	100.0(47/47)	66.7(2/3)	88.9(112/126)	96.6(28/29)	0.02

Chloramphenicol	72.3(704/974)	89.2(173/194)	95.9(372/388)	82.9(68/82)	100.0(1/1)	-	29.2(90/308)	0.0(0/1)	0.01
Colistin	1.7(4/230)	0.0(0/16)	0.0(0/2)	2.5(1/40)	2.6(1/38)	-	0.0(0/116)	0.0(0/18)	0.03
Cotrimoxazole	73.8(2,547/ 3,451)	89.2(173/194)	95.9(372/388)	82.9(68/82)	100.00(1/1)	-	29.2(90/308)	0.0(0/1)	<0.001
Pseudomonas aeruginosa	**% (n/N)****								
Imipinem	46.7(334/715)	31.0(36/116)	57.6(83/144)	55.6(15/27)	59.12(107/181)	29.4(15/51)	24.5(24/98)	55.1(54/98)	<0.001
Meropenem	44.4(319/718)	27.5(33/120)	61.2(93/152)	59.3(16/27)	58.1 (104/179)	33.3(9/27)	18.6(19/102)	44.1(45/102)	<0.001
Ceftazidime	20.1(287/1,431)	10.4(30/288)	21.2(89/419)	9.7(10/103)	39.5(118/299)	33.3(9/27)	13.1(17/130)	8.5(14/165)	0.001
Cefepime	36.0(367/1,021)	25.3(41/162)	36.6(108/295)	18.9(11/58)	45.8(120/262)	32.6(15/46)	24.3(26/107)	50.6(46/91)	<0.001
Piperacillin-tazobactam	31.9(452/1,419)	13.1(36/264)	29.5(118/400)	13.6(14/103)	64.0(178/278)	18.5 (12/65)	33.3(40/120)	30.2(54/179)	<0.001
Gentamicin	34.3(461/1,343)	19.8(49/249)	38.0(130/342)	28.7(27/94)	41.87(121/289)	26.3(15/57)	30.3(40/132)	43.9(79/180)	<0.001
Tobramycin	48.2(364/ 755)	32.2(47/146)	57.2(123/215)	42.3(22/52)	79.5(58/73)	26.5(13/49)	25.2(30/119)	70.3(71/101)	<0.001
Amikacin	29.4(236/804)	19.4(31/160)	32.6(70/215)	50.0(22/44)	45.2(56/124)	18.8(19/48)	21.4(22/103)	23.6(26/110)	<0.001
Ciprofloxacin	35.1(343/976)	21.43(39/182)	43.7(86/197)	46.9(30/64)	45.3(121/267)	29.7(11/37)	35.5(27/76)	18.9(29/153)	0.01
Colistin	1.9(4/212)	0.0(0/22)	4.6(2/44)	0.0(0/5)	0.0(0/107)	-	0.0(0/19)	13.3(2/15)	0.01

*Susceptibility suppression pattern at certain sites under reported resistance to cloxacillin.

CMJAH = Charlotte Maxeke Johannesburg Academic Hospital; SBPAH = Steve Biko Pretoria Academic Hospital; CHB = Chris Hani Baragwanath Hospital; HJ = Helen Joseph Hospital; UH = Universitas Hospital; GSH = Groote Schuur Hospital; TH = Tygerberg Hospital. ** % (n/N) proportion of resistant isolates (number resistant/total number of isolates tested;

Chi square p-value of independence showing significant difference in resistance between year of testing.

Data from CMJAH, SBPAH, CHB and HJ for cloxacillin resistance were unreliable, as NHLS laboratories in Gauteng province had systematic error in reporting MRSA.

5.3.5 Trends of *S. aureus* resistance

The total number of reported cloxacillin-resistant SA declined progressively from 182 to 91 during the period 2006 to 2009 as did the ratios of resistant to susceptible isolates (expressed in percentages). A decline in MRSA in recent years has also been reported in Scottish and European hospitals due to rigorous infection control measures such as simple hand washing before touching patients, eating food and after using the toilet. The total numbers of resistant isolates of SA, but not the ratios of resistant to susceptible cultures, also showed steady declines in the case of gentamicin, clindamycin and rifampicin resistance. (Table 5.4)

Table 5.4 Trends of antimicrobial resistance rate of selected blood borne infections by year

Antibiotics tested	Total	2005	2006	2007	2008	2009	p-value
Staphylococcus aureus	% (n/N) **						
Ampicillin	95.7(3,322/3,471)	96.2(305/317)	95.9(869/906)	94.9(755/795)	95.6(688/720)	96.2(705/733)	0.774
Cloxacillin	15.4(588/3,828)*	22.2(74/334)	19.0(182/960)	14.5(123/848)	14.4(118/817)	10.5(91/869)	0.042
Vancomycin	0.1(1/865)	0.0(0/106)	0.4(1/288)	0.0(0/212)	0.0(0/145)	0.0(0/114)	0.735
Gentamicin	51.8(428/827)	53.6(59/110)	45.5(141/310)	46.5(87/187)	67.8(97/143)	57.14(44/77)	<0.001
Erythromycin	34.5(802/2,326)	40.1(95/237)	40.3(242/601)	29.6(182/614)	31.2(148/475)	33.8(135/399)	<0.001
Clindamycin	32.4(650/2,005)	37.1(79/213)	38.0(194/511)	26.3(145/552)	32.2(125/388)	31.4(107/341)	<0.001
Rifampicin	39.9(194/486)	32.0(24/75)	35.5(59/166)	34.6(44/127)	66.7(46/69)	42.9(21/49)	<0.001
Fusidic acid	8.5(33/388)	11.7(8/68)	6.2(9/146)	5.3(6/114)	16.3(7/43)	17.7(3/17)	0.070
Cotrimoxazole	29.9(577/1,930)	33.3(67/201)	33.1(168/508)	24.9(128/514)	29.6(117/395)	31.1(97/312)	0.042
Klebsiella pneumonia	% (n/N) **						
Amoxiclav	64.8(2,550/3,936)	60.0(189/315)	60.1(527/877)	61.6(559/908)	70.1(629/897)	68.8(646/937)	<0.001
Imipinem	0.1(4/3,059)	0.4(1/255)	0.0(0/663)	0.0(0/653)	0.1(1/736)	0.3(2/732)	0.377
Meropenem	0.2(5/3,046)	0.0(0/255)	0.0(0/683)	0.3(1/669)	0.1(1/714)	0.4(3/725)	0.363
Ertapenem	2.0(50/2,474)	2.4(4/166)	2.7(11/410)	2.7(14/529)	1.2(8/665)	1.9(13/704)	0.350
Ceftazidime	82.0(2,428/2,962)	77.2(180/233)	79.0(512/648)	78.0(533/683)	85.5(591/691)	86.6(612/707)	<0.001
Cefuroxime	73.8(2,437/3,301)	65.5(188/287)	70.2(512/729)	69.3(516/745)	79.3 (593/748)	79.3(628/792)	<0.001
Cefoxitin	52.0(357/687)	13.9(11/19)	35.7(51/143)	62.7(133/212)	69.4(120/173)	52.5(42/80)	<0.001
Cefotaxime-ceftriaxone	66.0(2,238/3,390)	55.5(127/229)	56.9(376/661)	62.4(498/798)	72.2(618/856)	73.2(619/846)	<0.001
Cefepime	79.8(2,364/2,963)	73.2(164/224)	76.5(484/633)	76.6(518/676)	84.7(599/707)	82.9(599/723)	<0.001
Piperacillin-tazobactam	66.3(1,820/2,745)	58.6(123/210)	61.7(383/621)	62.5(422/675)	72.3(457/632)	71.7(292/407)	<0.001
Gentamicin	58.7(2,242/3,820)	52.4(176/336)	53.9(498/923)	51.5(468/909)	66.5(535/805)	66.7(565/847)	<0.001
Tobramycin	80.4(1,564/1,946)	75.8(116/153)	81.3(377/464)	79.3(318/401)	82.8(360/435)	79.7(393/493)	0.375
Amikacin	26.4(695/2,631)	21.2(54/255)	22.3(152/683)	32.0(197/615)	25.9(138/531)	28.2(154/547)	<0.001
Ciprofloxacin	51.8(1,380/2,666)	32.6(69/212)	40.5(231/570)	48.7(299/614)	58.5(397/679)	64.9(384/591)	<0.001
Chloramphenicol	72.3(704/974)	62.8(81/129)	69.2(189/273)	67.2(160/238)	67.2(158/208)	92.1(116/126)	<0.001
Colistin	1.7(4/230)	3.6(1/28)	0.0(0/22)	7.7(1/13)	0.0(0/13)	1.3(2/154)	0.401
Cotrimoxazole	73.8(2,547/3,451)	67.5(201/298)	69.7(556/798)	69.1(547/792)	77.5(605/781)	81.6(638/782)	<0.001
Pseudomonas aeruginosa	% (n/N) **						
Imipinem	46.7(334/715)	47.5(29/61)	31.3 (47/150)	47.3(79/167)	46.4(70/151)	58.6(109/186)	<0.01

Meropenem	44.4(319/718)	48.2(27/56)	27.5(42/153)	45.2(76/168)	47.8(76/159)	53.9(98/182)	<0.01
Ceftazidime	20.1(287/1,431)	17.1(21/123)	11.8(39/330)	21.5(73/339)	23.5(69/294)	24.6(85/345)	<0.01
Cefepime	36.0(367/1,021)	36.6(34/93)	26.3(55/209)	32.1(87/271)	41.5(85/205)	43.6(106/243)	<0.01
Piperacillin-tazobactam	31.9(452/1,419)	13.1(36/264)	29.5(118/400)	13.6(14/103)	64.0(178/278)	18.5 (12/65)	<0.01
Gentamicin	34.3(461/1,343)	39.0(48/123)	21.7(73/336)	37.5(50/133)	45.6(108/237)	36.4(107/294)	<0.01
Tobramycin	48.2(364/755)	38.7(24/62)	40.2(68/169)	60.2(112/186)	54.9(89/162)	40.3(71/176)	<0.01
Amikacin	29.4(236/804)	25.7(18/70)	16.2(29/179)	31.8(62/195)	30.7(60/163)	34.0(67/197)	<0.01
Ciprofloxacin	35.1(343/976)	25.9(28/108)	21.3(49/230)	36.3(86/237)	36.8(77/209)	53.7(103/192)	<0.01
Colistin	1.9(4/212)	0.0(0/7)	4.6(1/22)	0.0(0/56)	1.9(1/54)	2.7(2/73)	0.67

** % (n/N): proportion of resistant isolates (number resistant/total number of isolates tested

5.3.6 Demographic factors associated with *S. aureus* resistance

The age-group <5 years was significantly associated with SA resistance to antimicrobials. Children <5 years were 74% more likely to have had incidence of SA resistant isolates (AOR 1.74, CI 1.33-2.28) compared to the 20-29 years age-group. There was a significant association between antimicrobial resistance and hospital location. SA isolates at UH were three times more likely to be resistant to antimicrobials, (AOR 3.08. CI 2.10-4.52); SA isolates from Groote Schuur hospital were appreciably more likely to be resistant to antimicrobials (AOR 3.78, CI 2.85-5.01). At Tygerberg hospital, SA isolates were 4.8 times more likely to be resistant to antimicrobials (AOR 4.75, CI 3.60-6.20). In general SA isolates from UH, GSH, TH were significantly more likely to be resistant to antimicrobials. (Table 5.5)

5.3.7 Patterns of *K. pneumoniae* resistance

For the 5-year study period, the carbapenems covered the widest range of *K. pneumoniae* isolates. Cephalosporin resistance in KP was high but varied widely e.g. cefotaxime/ceftriaxone resistance was 50.0% - 65.1% for five of the seven hospitals, 83.7% and 92.5% for the other remaining two hospitals. Cefepime resistance was high in the three Johannesburg hospitals (92.5% - 94.4%) compared to 55.75 – 84.0% for the other remaining four hospitals.

Carbapenems and colistin resistance for KP shows to be still very low: imipenem and meropenem resistance at 0.1% each and ertapenem at 2.0%; colistin resistance at 1.9% while resistance rates for co-amoxiclav in KP isolates averaged at 64.8% for the seven hospitals while resistance to piperacillin-tazobactam with two exceptions were >65%. At four of the

seven hospitals, resistance to cotrimoxazole was in excess of 70%. The mean resistance rates for aminoglycosides were amikacin 26.4%, gentamicin 58.7% and tobramycin 80.4%.

Ciprofloxacin resistance rates in KP at the seven hospitals were ~50%. Piperacillin-tazobactam resistance in KP was high with a mean resistance of 66.3% and rates varying from 29.6% at GSH to 83.9% at CHB. (Table5.3)

5.3.8 Trends of *K. pneumoniae* resistance

There is a marked rise of ciprofloxacin resistance (32.6% in 2005 to 64.9% in 2009, p<0.001) and cotrimoxazole resistance (67.5% in 2005 to 81.6% in 2009, p<0.001). High rates of cephalosporin resistance maintained or slight increases seen over the 2005 to 2009 period, e.g. ceftazidime resistance 77.2% - 86.6%; cefotaxime (55.5% - 73.2%). There were high rates of aminoglycoside resistance showing a slight rise of resistance over this period, i.e. amikacin 21.2 - 28.2 %; gentamicin 52.4 - 66.7%; tobramycin 75.8 - 79.7%. There were significant differences in rate of KP resistance by year of study among most of the antibiotics except for carbapenems, nitrofurantion, tobramycin and colistin p>0.05. (Table 5.4)

5.3.9 Demographic factors associated with *K. pneumoniae* resistance

For KP, age-group <5 years was significantly associated with antibiotic resistance with children <5 years being 49% more likely to have KP resistant isolates (AOR 1.49, CI 1.19 - 1.88) compared to the 20 - 29 years age-group. Females were more likely to have resistant KP isolates than males (AOR 1.13 CI 1.00 - 1.29). There was a significant association between antimicrobial resistance and hospital location. KP isolates at UH were 39% more likely to be resistant to antimicrobials (AOR 1.39, CI 1.01 - 1.91); even though KP isolates from HJ, GSH and TH were more likely to be resistant to antimicrobials, this was not

statistically significant hence not reported in detail here. KP isolates reported in 2008 and 2009 were more likely to be resistant to antimicrobials; however this was not statistically significant. (Table 5.5)

5.3.10 Patterns of *P. aeruginosa* resistance

The mean ceftazidime resistance rate in PA was 20.1% and 36.0% for cefepime. Carbapenem resistance in PA was 46.7% and 44.4% respectively for imipenem and meropenem and 31.9% for piperacillin-tazobactam. The antibiotic with the greatest spectrum of activity against *P. aeruginosa* for the study period was colistin with a resistance rate of 1.9% (range 0% - 13.3%). Colistin resistance was absent in PA in four of the seven hospitals where susceptibility testing was performed. The mean ciprofloxacin resistance was 35.1% and for amikacin, gentamicin and tobramycin resistance rates in PA were 29.4%, 34.3% and 48.2% respectively. (Table 5.3)

5.3.11 Trends of *P. aeruginosa* resistance

The range of ciprofloxacin resistance was (25.9% - 53.7%) over the period 2005 – 2009; moderate increases in aminoglycoside resistance among PA isolates over the study period were observed (amikacin 25.7% – 39.1%, gentamicin 21.7% – 53.7%, and tobramycin 38.7% - 60.2%). Cephalosporin resistance equally showed moderate rise i.e. ceftazidime 17.1% - 24.6% and cefepime 36.6% – 43.6%. Carbapenems resistance rate showed moderate rises ~45% - ~55% for imipenem and meropenem resistance over the 2005 – 2009 periods. (Table 5.4)

5.3.12 Demographic factors associated with *P. aeruginosa* resistance

For the 5-year study period, hospital location was associated with antibiotic resistance. SBPAH (AOR 5.16, CI 3.62 - 7.36), GSH (AOR 2.08, 1.35 - 3.21), TH (AOR 3.02, 2.04 - 4.47) were significantly associated with antibiotic resistance among *P. aeruginosa* isolate*s*. At UH, PA isolates were 60% more likely to be resistant to antimicrobials; however this was not statistically significant (AOR 1.60, CI 0.91 - 2.79). Even though PA isolates reported in 2008 and 2009 were more likely to be resistant to antimicrobials, the association was not statistically significant. (Table 5.5)

Table 5.5 Univariate and multivariate analysis of factors associated with antimicrobial drug resistance among selected blood culture infections

Characteristic	*Staphylococcus aureus*		*Klebsiella pneumonia*		*Pseudomonas aeruginosa*	
	UOR (95% CI)	AOR (95% CI)	UOR (95% CI)	AOR (95% CI)	UOR (95% CI)	AOR (95%CI)
Age						
<5	1.01(0.79-1.27)	1.74(1.33-2.28)	1.51(1.21-1.88)	1.49(1.19-1.87)	0.87(0.63-1.20)	0.83(0.58-1.19)
5-9	0.52(0.29-0.93)	0.66(0.35-1.26)	1.70(0.93-3.12)	1.58(0.86- 2.91)	1.19(0.58- 2.44)	1.28(0.60-2.74)
10-19	0.79(0.56-1.11)	0.84(0.56-1.25)	1.01(0.71-1.42)	0.94(0.66-1.34)	0.96(0.56- 1.67)	0.95(0.53-1.70)
20-29	1 (0.79-1.27)	1	1	1	1	1
30-39	1.19(0.96-1.48)	1.25(0.94-1.67)	1.17(0.905-1.50)	1.15(0.89-1.48)	1.26(0.87-1.82)	1.25(0.85-1.85)
40-49	0.90(0.70-1.15)	0.93(0.68-1.27)	0.89(0.68- 1.16)	0.89(0.68- 1.16)	0.92(0.62-1.36)	0.91(0.60-1.38)
50-59	0.82(0.62-1.09)	0.80(0.56-1.13)	0.90(0.68-1.18)	0.88(0.66-1.16)	1.00(0.66-1.51)	0.97(0.62-1.50)
60-69	0.93(0.68-1.27)	0.96(0.66-1.39)	0.95(0.71-1.27)	0.92(0.69-1.23)	1.08(0.70-1.67)	1.08(0.67-1.72)
≥70	0.68(0.46-1.00)	0.78(0.50-1.23)	0.82(0.60-1.11)	0.78(0.57 -1.07)	1.02(0.63-1.66)	1.01(0.61-1.70)
Gender						
Male	1	1	1	1	1	1
Female	0.99(0.85-1.15)	0.97(0.82-1.14)	1.17(1.03-1.33)	1.13(1.00-1.29)	1.03(0.84-1.27)	0.99(0.80-1.24)
Hospital**						
CMaxeke JAH	1	1	1	1	1	1
CHani Bara	0.41(0.31-0.55)	0.41(0.30-0.56)	1.22(1.01-1.48)	1.08(0.89-1.32)	1.92(1.40-2.62)	1.87(1.34-2.62)
Helen Joseph	0.95(0.68-1.33)	1.26(0.88-1.80)	1.09(0.81-1.46)	1.23(0.91-1.67)	1.66(1.05-2.65)	1.48(0.91- 2.39)
SBPAcademic	0.83(0.60-1.15)	1.00(0.72-1.41)	0.95(0.77-1.18)	0.95(0.76-1.18)	5.43(3.84-7.68)	5.16(3.62-7.36)
Universitas	2.55(1.76-3.70)	3.08(2.10-4.52)	1.56(1.14-2.134)	1.39(1.01-1.91)	1.80(1.04-3.11)	1.60(0.91-2.79)
Groote Schuur	2.98(2.29- 3.89)	3.78(2.85-5.01)	1.04(0.82-1.31)	1.10(0.86-1.39)	2.33(1.53- 3.55)	2.08(1.35-3.21)
Tygerberg	4.10(3.18-5.29)	4.75(3.6- 6.20)	1.25(0.99-1.58)	1.12(0.88-1.42)	3.20(2.18-4.70)	3.02(2.04-4.47)

Province

Gauteng	1		1	---	1	---
Free State	3.69(2.65-5.12)	---	1.44(1.09-1.90)	---	0.85(0.51-1.40)	---
Western Cape	5.13(4.37- 6.02)	---	1.05(0.91-1.21)	---	1.32(1.03-1.69)	---

Year

2005	1	1	1	1	1	1
2006	0.82(0.63-1.07)	0.72(0.54-0.97)	0.93(0.72-1.20)	0.90(0.69-1.17)	0.48(0.32-0.72)	0.52(0.33-0.80)
2007	0.67(0.51-0.89)	0.61(0.45-0.82)	0.88(0.68-1.13)	0.87(0.67-1.13)	0.84(0.56-1.25)	0.79(0.51-1.21)
2008	0.56(0.43-0.74)	0.48(0.35-0.65)	1.27(0.99-1.64)	1.29(0.99-1.68)	1.21(0.81-1.80)	1.20(0.78-1.84)
2009	0.44(0.33-0.58)	0.39(0.28-0.53)	1.27(0.99-1.65)	1.27(0.98-1.66)	1.13(0.76-1.68)	1.11(0.72-1.70)

CI, confidence interval; UOR, unadjusted odds ratio; AOR, adjusted odds ratio ** CMaxeke JAH: The reference hospital

5.4. Discussion

This study led to a detailed and systematic data analysis of the LIS in reporting antimicrobial susceptibility of isolates from blood culture over a 5 year period, to assess possibility for reporting of trends and patterns of resistance from all isolates in public tertiary hospitals in South Africa. A total of 9969 isolates were identified belonging to *S. aureus*, *K. pneumoniae* and *P. aeruginosa* had drug susceptibility results reported on by the NHLS between July 1, 2005 and December 31, 2009. The numbers of isolates of all three pathogens for 2005 (first year of CDW-based surveillance) were substantially smaller than the other years, as the surveillance system started half way through that year.

S. aureus and *K. pneumoniae* were the most common pathogens and contributed 84.3% of the total magnitude of blood stream infections among the three selected pathogens reported within this period. This is in keeping with previous studies that have shown *S. aureus* to be the predominant cause of blood stream infections. (115, 116, 124) More isolates of these pathogens were reported from males and children below the age of 5 years. The relationship of higher incidence of blood stream infections among males has been documented in previous studies. (115, 125) As much as this study found higher incidence of blood stream infections among children, other studies in Canada and the USA have found smaller proportion of isolates from children. (126) There were more isolates reported from Chris Hani Baragwanath Hospital, which is the largest hospital in the country and services a historically disadvantaged population of Soweto. Antimicrobial susceptibility was done to assess rates of resistance to various antibiotics amongst the three common pathogens associated with in-hospital acquisition.

The proportion of *K. pneumoniae* resistant isolates (defined as isolates resistant to one or more antibiotics) was higher among females while *S. aureus* and *P. aeruginosa* rates were similar. The proportion of *S. aureus* resistant isolates was highest at Tygerberg Hospital, *K. pneumoniae* was highest at Universitas Hospital and *P. aeruginosa* was highest at Steve Biko Pretoria Academic Hospital. There were more resistant isolates of *S. aureus* and *P. aeruginosa* reported from the Western Cape and more resistant isolates for *K. pneumoniae* reported from Free State province. The proportion of *S. aureus* resistant isolates was higher in 2005; *K. pneumoniae* and *P. aeruginosa* were higher in 2008.

No recent studies in South Africa on the frequency of bacteraemic pathogens have documented comparable information. This study used blood culture data that represent invasive pathogens and therefore excludes organisms that merely colonize non-sterile sites and may be present in specimens such as pus swabs. Such data could serve to guide prescription habits and form the basis of a robust national surveillance monitoring system able to regularly document similarities and differences in antimicrobial resistance between different hospitals both locally and internationally.

Antibiotics with the broadest spectrum against *S. aureus* were vancomycin and linezolid. Vancomycin was still active against nearly all *S. aureus* isolates with resistance rate showing <0.1% across all the 7 hospitals. This is consistent with previous data which reported that vancomycin was still an active agent against *S. aureus* including MRSA.(31, 127-130) Frequencies of clindamycin resistance (72%), erythromycin resistance (44.1%) and rifampicin (60.7%) among *S.aureus* isolates at TH are relatively high. These might be linked to macrolide or as a result of inducible clindamycin resistance among erythromycin resistant strains. Simultaneous resistance to erythromycin and clindamycin among *S.aureus* isolates

could be a result of erythromycin resistance methylase genes (*erm* genes), while erythromycin resistance not crossed to clindamycin is consistent with the presence of *msrA* gene. The variation in susceptibility of erythromycin-resistant *S.aureus* to clindamycin as observed in this study among the seven tertiary public hospitals might be an indication of epidemiological variation in the two mechanisms of resistance that was mentioned above. (131, 132)

The highest rates of MRSA were observed at Tygerberg and Groote Schuur hospitals in the Western Cape as opposed to Universitas hospital in the Free State province. In Gauteng province hospitals, MRSA rates of 0.4% - 4.5% were observed, questioning the reliability of such findings and this made it difficult in this study to make any meaningful comparison of resistance rates with other sites. It may also be because there is a bias in culturing more community acquired infections than hospital acquired. The variation in rates of MRSA observed is consistent with previous EARSS reports(125) that showed marked geographical variation in prevalence of MRSA. In the current setting, the plausible explanation for this variation might be due to differences in specimen collection, carriage rates or hospital infection control policies and practices as well as prescription policies between different hospitals and provincial Departments of Health. (115)

The other reason might relate to differences in laboratory practices between different sites, with NHLS laboratories in Gauteng province failing to report cloxacillin resistant isolates, as opposed to NHLS laboratories in Western Cape and Free State. However, despite the geographical differences in MRSA and the observed systematic error in MRSA reporting in Gauteng hospitals, overall there was an apparent decline in MRSA in this province which on calculation was statistically significant (from 22.2 % (74/334) in 2005 to 10.5% (91/869) in

2009, p<0.042. However, without clinical information, this observed trend may be assumed to flawed, hence not real. On the other hand this finding is consistent with the EARSS report that documented that more countries within the Pan-European antimicrobial resistance surveillance showed decreasing MRSA proportions even though the rates still remained at >25% in almost one third of the countries. (125) In the UK, a national surveillance scheme run by the HPA, observed decreasing rates of MRSA from 31% in 2007 to 19.3% 2009 (133). In addition, a similar trend was observed in Canadian hospitals, where Adam et al reported a drop in MRSA rates from 26.7% in 2007 to 18.9% in 2006. (115) Based on available evidence highlighted earlier, the observed trend of MRSA decline appears to be consistent with observed global trends. This correlates well with initiatives from Departments of Health to introduce strict infection control measures and mandatory surveillance for MRSA.

In light of these findings, the reliability of routine laboratory data generated by the LIS for monitoring antimicrobial resistance requires further interrogation, as it remains unclear if the observed rates of antimicrobial resistance are realistic and not due to selection bias. On a different note, it is worthwhile to reassess and scrutinize the validity of the observed finding. Such differences might have been due to multiple factors, among them strengthening of hospital infection control policies and antimicrobial stewardship; training and implementation of hand-washing hygiene or changing epidemiology of MRSA in South Africa over the study period.

The most active antibiotics against *K. pneumoniae* in this study were the carbapenems. These data are similar to those shown by Zhanel et al. (130) Cephalosporin, flouroquinolones and aminoglycosides showed high resistance across all sites. β-lactams, excluding carbapenems

were the least active antibiotics over the 4.5-year study period with resistance rate increasing in all sites and in keeping with previous review findings done in South Africa. (31) Low levels of carbapenem resistance, shows that there is evidence of emergence of carbapenemase-mediated resistance among KP isolates. Nordmann et al reported that *K. pneumoniae* that produces K.*pneumoniae* carbapenemase (KPC) have globally spread across hospitals. (134) However, it is a growing concern to note emerging colistin resistant KP. Bogdanovich et al, reported cases of KP-carbapenemase producing isolate that showed emerging resistance to colistin. (135) This is a worrying development as colistin is the last line of defence; it is reserved for treatment of severe Gram negative sepsis that has resulted from failed treatment with carbapenems. This is reassuring, as carbapenems have been shown in a multicentre study to have the most favourable outcomes in the treatment of bacteraemic ESBL-producing KP infections. (136) There was a significant trend of KP resistance to ciprofloxacin and cotrimoxazole, while meropenem showed a significant increasing trend of resistance from 2006 to 2009 - no particular resistance trend was observed for other antibiotics. (127)

P. aeruginosa resistance was evident across most of the drug classes, showing high resistance to carbapenems, cephalosporin, flouroquinolones and aminoglycosides. Carbapenem resistance in *P. aeruginosa* is often mediated through genetic down regulation of outer membrane protein D. Even though Adam et al., in a study done among Canadian hospitals reported that resistance was encroaching to these drug classes, the resistance rate shown in this study, is far higher compared to the findings of Adam et al. This is a significant finding denoting that geographical location does play a role in development of antimicrobial resistance, and therefore might mean that due to rapid increase and high level of

intercontinental mobility, resistant clones are bound to spread across different countries and regions. (115, 127, 137)

The rates of aminoglycoside resistance among *P. aeruginosa* and *K. pneumoniae* isolates was varied with amikiacin showing low resistance and tobramycin showing higher resistance. As shown above, among *K.pneumoniae* isolates, the mean resistance for amikacin was 26.4%, gentamicin 58.7% and tobramycin 80.4%, whereas *P. Aeruginosa*, the mean resistance for amikacin, gentamicin and tobramycin were 29.4%, 34.3% and 48.2% respectively. Such observed differences in resistance patterns could be due to differences in aminoglycoside modifying enzymes; prescription patterns or variation in quality of infection control practices in these hospitals, although geographical differences in the occurrence of individual aminoglycoside resistance determinants might also play a role. This emphasizes the fact that the prudent use of aminoglycosides as well as implementation of effective infection control practices are essential in limiting the development and continued spread of aminoglycoside resistance among these pathogens. (138) The only consistently active antibiotic against *P. aeruginosa* for the study period was colistin, which had resistance rate of 1.9%. This is similar to findings of previous studies that also showed a similar pattern of high activity of colistin against *P. aeruginosa*. (127, 139)

Several demographic factors were found to be significantly associated with antimicrobial resistance. For SA, factors were: age-group <5 years; hospital location (UH, TH, GSH) and year of infection. Factors associated with KP resistance were age-group <5 years, female gender and hospital location (UH). The only factor significantly associated with PA resistance was hospital location (CHB, SBPAH, GSH and TH). There was however no data from our study that could explain such underlying associations despite the fact that environmental reservoirs and magnitude of burns of patients in hospitals are among the known drivers of *P. aeruginosa* resistance

5. 5 Limitations of the study

This study had several limitations which are related to the analysis of routine laboratory data. No clinical data were available; hence any determination of the impact of antimicrobial resistance on clinical outcomes could not be made. Such data are essential as their availability would help in making detailed risk factor analysis, evaluating the potential impact of inappropriate antimicrobial therapy on outcome of patients with bacteraemia episode caused by the three selected pathogens.

Secondly, the magnitude of blood stream infection caused by the selected pathogens was not determined. Such data would be useful to give precise estimates of the magnitude of blood stream infection caused by such organisms, as this would help direct strategic planning of service delivery, medication procurement as well as intensity of hospital infection control procedures.

Thirdly susceptibility testing methods for individual antibiotics varied across sites for individual pathogens, with other NHLS laboratories testing certain specific agents more than other sites, which might have led to differences in estimation of resistance rates among those agents. Fourthly while using the first specimen only is one approach to surveillance, a limitation of such an approach is the possibility of missing the occurrence of acquired resistance during the illness. This may not be captured by the surveillance system. In addition although susceptibility testing figures were used to assess rates of resistance among the three pathogens associated with hospital acquisition, our results do not differentiate between community and hospital acquired infections and there is therefore the potential for underestimation of resistance rates in general.

Another important limitation is that no data was available on admission date for each patient and specimen collection hence no accurate description of community versus nosocomial acquired bacteraemia could be made. Lastly the use of 'resistance to any antimicrobial agent' tested as a method of estimating overall resistance rate. This method might have led to erroneous estimation of resistance among the antibiotics tested as shown in Table 5.5.

5.6 Conclusion

There are problems in retrieving information on AST from the current LIS. Estimated rates of antimicrobial resistance observed in this study, are a matter of grave concern, especially with regard to PA and KP. It was encouraging to see that other antimicrobial agents are still very active against the selected pathogens.

Firstly, the rate of vancomycin resistance is almost negligible (0.1%, only 1 of 865 isolates- one case at Tygerberg hospital in 2006) and linezolid resistance among *S. aureus* isolates was not detected in this study. Secondly, carbapenems (ertapenem, imipenem and meropenem) and colistin remains highly active against *K. pneumoniae* and thirdly, that colistin is highly active against *P. aeruginosa.* The extent of antimicrobial resistance in PA is alarming and is aggravated by the fact that colistin is both oto- and nephrotoxic.

Therefore ongoing structured prospective surveillance to monitor the burden of bloodstream infections and their resistance profile is essential to better monitor trends and patterns of resistance to nosocomial infections at national level. Such data would enhance the knowledge of the magnitude of the problem regarding antimicrobial resistance and will form evidence upon which policies and practice aimed at containing antimicrobial resistance can be generated. In addition the analysis presented in this chapter provides the type of assessment that has to be used to develop empirical treatment guidelines.

Chapter 6   Distribution and risk factors of antimicrobial resistance of invasive *Staphylococcus aureus* and *Klebsiella pneumoniae* blood culture isolates from seven academic hospitals in South Africa-a prospective study

This chapter provides findings of prospective analysis of antimicrobial resistance data of clinical isolates of *Staphylococcus aureus and Klebsiella pneumoniae* cultured from blood of patients presenting to hospital with bacteraemia episodes. The aim was to compare rates of resistance from prospective data with rates obtained from retrospective data with the view of finding out reliability of the LIS as a tool for monitoring antimicrobial resistance patterns in tertiary public hospitals in South Africa.

6.0 Abstract

Aim: To describe antimicrobial resistance profiles and risk factors of blood culture isolates of *Staphylococcus aureus* (SA) *and Klebsiella pneumoniae* (KP) from seven academic hospitals in South Africa, using data of bacterial isolates collected prospectively through an active national antimicrobial resistance surveillance system.

Methods: Blood-culture isolates of SA and KP were detected and identified by automated MicroScan, Vitek 2 systems or standard biochemical tests. Antimicrobial susceptibility testing was done following manufacturers' instructions and interpreted using the Clinical Laboratory Standards Institute (CLSI) guidelines. The identified blood culture isolates were systematically investigated for resistance against clinically relevant antimicrobials.

Results: There were 3026 isolates reported between July 2010 and June 2011; of these 1494 (49.4%) were SA and 1532 (50.6%) were KP. Of the SA and KP isolates 68.0% and 71.1% respectively were from Gauteng province. The rate of SA resistance to methicillin (MRSA) was 558/1032 (54.1%) but it was higher (63.3%) in the <5 years age-group, and significantly different across all hospitals ranging between 31.8%-63.3% (p=<0.001). The highest rates of MRSA (243/292, 83.2%) were observed at Chris Hani Baragwanath (CHB) hospital, Gauteng province. SA resistance rates among fusidic acid, vancomycin among others were on average <1.5%, suggesting an infiltration development of resistance to these antimicrobial agents.

There were (742/1045, 71.0%) extended spectrum beta-lactamase producing KP (ESBLs-KP) isolates. The <5 years age-group had the largest number of ESBL-KP isolates (266/340, 78.2%) and there were significant differences in ESBL production between different age-groups, p = 0.003. KP resistance to carbapenems, ranged from 1.3 - 3.4% and to other extended spectrum cephalosporins such as cefepime, resistance was high at 70.4% which is in accordance with the high proportions of ESBL-producing isolates recorded in this study. For betalactams i.e. amoxicillin/clavulanic acid, resistance ranged from 50.0 - 69.9% (p=0.007) across all hospitals. ESBL-KP was lowest at Steve Biko Pretoria Academic Hospital (SBPAH) and highest at Universitas Hospital (61.9 - 79.7%, P = 0.012). Overall there were significantly lower rates of MRSA (p = <0.001) and ESBLs (p = 0.021) at SBPAH compared to all other hospitals.

Conclusions: This study describes high rates of antimicrobial resistance among blood culture isolates of SA and KP from academic hospitals in South Africa. Continued surveillance of antimicrobial resistance would provide useful data for guidance to physicians initiating

empiric therapy, and for the formulation of antimicrobial prescription policies in South Africa.

6.1 Introduction

Antimicrobial resistance (AMR) amongst hospital and community-acquired bacterial infections is an important clinical and public health challenge globally. (140-142) Several factors have been attributed to the increasing frequency of resistance to antimicrobials. Among these are natural characteristics of microbes, selective pressure due to intensive antimicrobial use and an increase in globalization due to advances in transportation and telecommunications infrastructure, some of which facilitate the transmission of resistant bacteria. (140, 143) Blood stream infections, commonly hospital-associated, are frequently caused by *Staphylococcus aureus* (SA) and *Klebsiella pneumonia* (KP). This frequency of bacteraemic episodes caused by these two organisms can serve as a guide to the magnitude of nosocomial infections in different settings. (122, 140, 144)

The challenges encountered in managing nosocomial bacteraemia which are often severe infections in both developed and developing countries are complex and daunting. Increasing resistance to a wide array of conventional antibiotics often leads to increased morbidity and mortality due to the therapeutic failure of empirical treatment. (122, 145) Clinicians are not always aware of resistance patterns to common pathogens in their hospitals and patient treatment environment. Subsequent wrong treatment choices may lead to longer hospital stay, initiation of costly second-line antibiotic regimens and escalating medical expenditure. (122, 140, 146) Useful and reliable measurement of the burden of antimicrobial resistance has often been impeded by the lack of an organized system of blood culture data collection, different

strategies for taking of blood cultures and varying levels of resistance associated with different health care facilities. (144)

Surveillance of antimicrobial resistance provides objective information on the burden of resistance among bacterial pathogens such as SA and KP. However limitations of a passive system need to be taken into account when interpreting surveillance findings. Surveillance data accessible to key hospital personnel including molecular epidemiological investigation would assist in the prioritization and strategic planning of infection prevention interventions coupled with policy guidance on antimicrobial prescription. (16, 143, 147) This study aimed to describe the frequency, distribution and risk factors associated with resistance among SA and KP blood culture isolates in South Africa using, for the first time, data from an enhanced national antimicrobial resistance surveillance system that collects blood culture data in the designated surveillance sites in South Africa.

6.2 Methodology

6.2.1 Invasive Disease Surveillance

The Group for Enteric Respiratory and Meningeal pathogens Surveillance in South Africa (GERMS-SA) has since 2003 been running enhanced invasive disease surveillance in all 9 provinces of South Africa. (148) GERMS-SA conducts disease surveillance of invasive respiratory, meningeal, and enteric infections. In 2010, GERM-SA added an additional component to the surveillance system to look at antimicrobial resistance among nosocomial pathogens.

The Antimicrobial Resistance Surveillance and Research (ARSR) within the Centre for Opportunistic, Tropical and Hospital Infections at the National Institute of Communicable Diseases (NICD) collected blood culture isolates of SA and KP in 7 tertiary public hospitals associated with academic institutions from three provinces in South Africa. Details of participating sites included in this study have been reported in Chapter 5, section 5.2.2. (31) Isolates of SA and KP were transported to ARSR laboratory from each participating site on Dorset egg transport media and stored at -70^0 C until pathogen identification and determination of minimum inhibitory concentrations (MIC) were done. (149)

6.2.2 Study Design

Data and clinical isolates of the two above mentioned blood stream pathogens were collected prospectively and tested for antimicrobial resistance. All non-duplicate isolates of SA and KP were prospectively sent by the microbiology laboratories of the participating sentinel sites to the Antimicrobial Resistance Surveillance and Research (ARSR) unit on an ongoing basis for confirmation and further characterization. The present study focused mainly on clinically relevant blood stream infections that are commonly associated with stay in hospital. (150) These isolates were identified at each participating hospital laboratory through routine blood culture investigation and sent to the antimicrobial reference laboratory at NICD where further testing was done. The study was approved by the Human Research Ethics Committee, University of the Witwatersrand, approval number M10625 (Appendix 12.3.8).

6.2.3 Data collection

Clinical Isolates of SA and KP were confirmed at ARSR using the automated MicroScan system. Due to the volume of the isolates sent from the participating sentinel sites, isolates

were stored for a median time of 6 - 8 weeks before being processed. All data originating from processing of the isolates, confirmation of pathogens and antimicrobial susceptibility testing as well as molecular characterizations were double entered into an MS access database at ARSR, NICD, Sandringham. Additional data collected included patients' demographics (age and gender), hospital location, hospital ward, hospital name, province and year of collection. This study used data of SA and KP isolates collected between July 2010 and June 2011.

6.2.4 Susceptibility Testing

Antimicrobial susceptibility testing of the isolates was carried out using the broth microdilution method as described by the Clinical Laboratory Standards Institute (CLSI).(151) Microdilution assays were obtained from MicroScan (Sacramento, California) and Media Laboratories (Tualantin, Oregon). Antibiotics tested were ciprofloxacin (SA and KP), clindamycin (SA), erythromycin (SA), fusidic acid (SA), gentamicin (SA and KP), rifampicin (SA), vancomycin (SA), oxacillin (SA), trimethoprim/sulfamethaxazole (SA and KP), ampicillin (SA and KP), cefazolin (SA and KP), cefuroxime (SA and KP), ertapenem (SA and KP), gentamicin (SA and KP), imipinem (SAand KP), meropenem (SA and KP), levofloxacin (SAand KP), tobramycin (SA and KP), amikacin (KP), cefepime (SA and KP), cefotaxime (KP), cefuroxime (KP), ceftazidime (KP), amoxicillin-clavulanate (SA and KP), piperacillin-tazobactam (KP), linezolid (SA), tetracycline (SA and KP), and tigercycline (KP). (113, 152)

6.2.5 Quality Control

The quality control of reagents used for the purpose of susceptibility test of isolates submitted from participating sentinel sites was evaluated by confirmatory testing carried out by the

ARSR laboratory using ATCC QC organisms: SA ATCC 29213 and KP 700603 routinely as control organisms. (113, 127) Interpretation of MICs breakpoints for each antibiotic tested was defined according to the CLSI guidelines. (151)

6.2.6 Statistical analysis

"Intermediate" and "resistant" isolates were grouped together into a "non-susceptible" category to create a binary variable called 'non-susceptible' indicating the presence or absence of antimicrobial resistance. Exposure variables included in the analysis were age, gender, hospital name, year of infection and province. The prevalence of resistance was estimated using percentages. Associations between resistance and various exposures were assessed using chi-squared test for independence. Missing data were excluded from further bivariate analysis. All analyses were done using Stata version 12 software (StataCorp Limited, College Station, Texas, USA).

6.3 Results

6.3.1 Distribution of *S. aureus* and *K. pneumoniae* isolates

A total of 1494 SA and 1532 KP isolates were analyzed for the period July 2010 to June 2011. Thirty point four percent of SA isolates and 32.3% of KP isolates were from patients under the age of five, 12.1% of SA isolates were from patients in the 30 - 39 years age-group and 11.8% of the KP isolates were from patients in the 50 - 59 years age-group; 11.3% of SA and 8.8% of KP isolates had missing data on age.

The proportion of isolates recorded from male patients was higher than females (50.0% vs. 42.6% for SA (7.4% missing) and 52.0% vs. 41.5% for KP (6.6% missing)). By institution, there were more SA and KP isolates reported from CHB (29.3% SA and 29.9% KP). Overall 67.9% and 71.0% of the SA and KP isolates respectively were from Gauteng province. Slightly more isolates were reported in 2010 and 2011 (SA 51.5% and KP 50.1% respectively. The proportion of missing data was observed among age and gender factor being higher amongst the age than gender factor. (Table 6.1)

Table 6.1 Distribution of *Staphylococcus aureus* and *Klebsiella pneumonia* isolates according to age, gender, hospital, province and year during prospective period.

Characteristics	*S. aureus* Frequency (N=1494)(%)	*K. pneumoniae* Frequency (N=1532)(%)
Age-group		
<5	454 (30.4)	494 (32.3)
5-9	32 (2.1)	14 (0.9)
10-19	61 (4.1)	53 (3.5)
20-29	145 (9.7)	128 (8.4)
30-39	180 (12.1)	169 (11.0)
40-49	141 (9.4)	147 (9.6)
50-59	135 (9.0)	181 (11.8)
60-69	97 (6.5)	128 (8.4)
>=70	80 (5.4)	84 (5.5)
Missing	169 (11.3)	134 (8.8)
Gender		
Male	747 (50.0)	796 (52.0)
Female	637 (42.6)	635 (41.5)
Missing	110 (7.4)	101 (6.6)
Hospital		
CHB	437 (29.3)	458 (29.9)
CMJAH	205 (13.7)	285 (18.6)
GSH	236 (15.8)	197 (12.9)
HJH	146 (9.8)	93 (6.1)
SBPAH	227 (15.2)	252 (16.5)
TH	143 (9.6)	137 (8.9)
UH	100 (6.7)	110 (7.2)
Province		
Free State	100 (6.7)	110 (7.2)
Gauteng	1015 (67.9)	1088 (71.0)
Western Cape	379 (25.4)	334 (21.8)
Year		
2010	770 (51.5)	764 (49.9)
2011	724 (48.5)	768 (50.1)

CHB - Chris Hani Baragwanath Hospital; **CMJAH** - Charlotte Maxeke Johannesburg Academic Hospital; **GSH** - Groote Schuur Hospital, **HJH** - Helen Joseph Hospital, **SBPAH** - Steve Biko Pretoria Academic Hospital, **TH** - Tygerberg Hospital, UH – Universitas Hospital.

6.3.2 Antimicrobial resistance pattern of SA and KP isolates

More than 50.0% of SA isolates were resistant to anti-staphylococcal beta-lactams (amoxicilin-clavulanate 54.6%, oxacillin 54.1%), carbapenems (imipenem 54.8%, ertapenem 54.9%, meropenem 55.0%) and cephalosporins (cefepime 54.5%). However, less than 1.5% of SA isolates were resistant to fusidic acid, synercid, teicoplanin and vancomycin. No isolates resistant to daptomycin or linezolid were identified. (Figure 6.1)
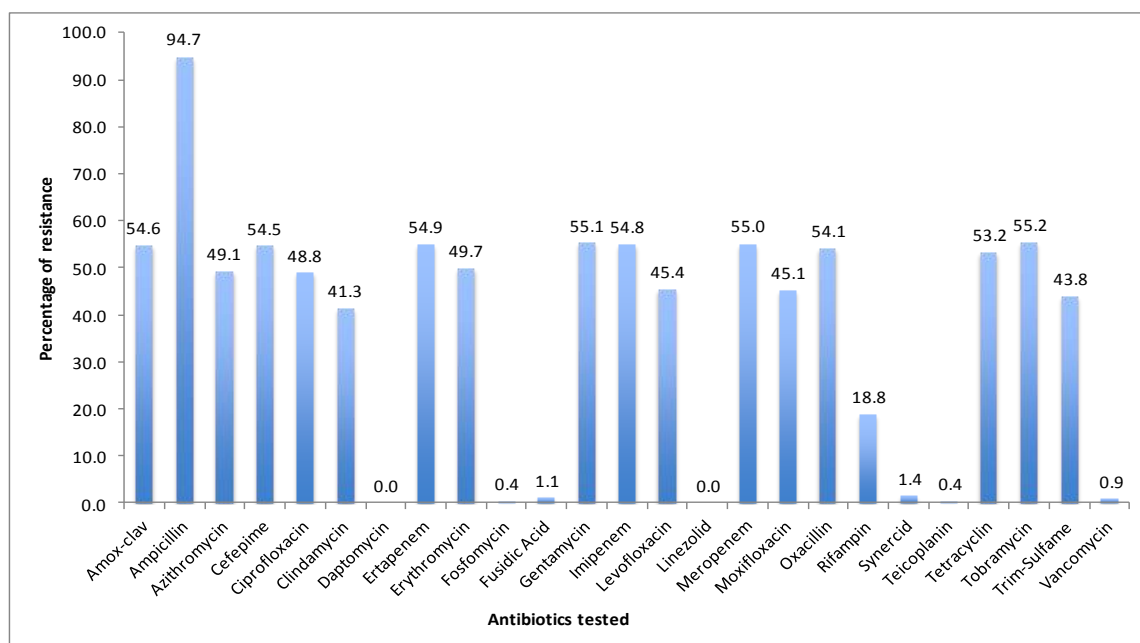
Figure 6.1 Profile of antimicrobial resistance of S.aureus

The proportion of KP isolates resistant to beta-lactams ranged between (30.1% and 79.3%): piperacillin-tazobactam 30.1%, amoxicilin-clavulanate 64.4%, and piperacillin 79.3%; resistance to carbapenems ranged between 1.3 - 3.4% and 70% of isolates were resistant to cephalosporins (cefepime 70.6%, cefotaxime 70.4%). Low resistance rates among KP isolates were observed to amikacin 4.5%, tigercycline 7.9 % and fosfomycin 8.8%. (Figure 6.2)
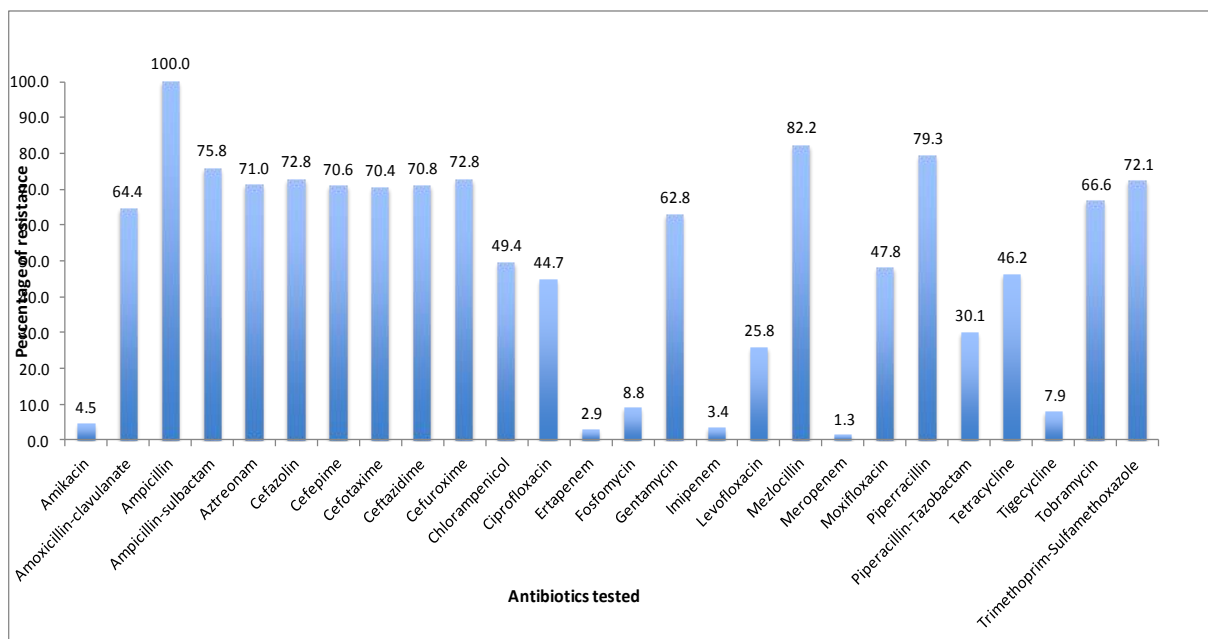
Figure 6.2 Profile of antimicrobial resistance of K.pneumoniae

6.3.3 Patterns of antimicrobial resistance rate by gender

Consistently higher proportions of SA isolates were resistant to various antibiotics among females compared to males and these differences were mostly statistically significant. Resistance rates were (females vs. males): beta-lactams (amoxicilin-clavulanate 56.9% vs. 49.8%, p = 0.030; oxacillin 56.7% vs. 49.0%, p = 0.019); carbapenems (imipenem 57.4% vs.50.0%, p = 0.024; ertapenem 57.2% vs.50.2%, p = 0.034; meropenem 57.2% vs. 50.6%, p = 0.045) and cephalosporins (cefepime 56.9% p = 0.026). For KP isolates, there was no consistent pattern in the proportions of resistant isolates to various antibiotics among females

compared to males. Resistance rates were: beta-lactams (piperacillin-tazobactam 34.4% vs. 27.4%, p = 0.021; amoxicilin-clavulanate 66.6% vs.62.9%, p = 0.233; piperacillin 78.9% vs.78.5%, p = 0.871); carbapenems (ertapenem 2.6% vs. 2.9%, p = 0.815; imipenem 2.8% vs. 3.4%, p = 0.609; meropenem 1.7% vs. 0.7%, p = 0.170) and cephalosporins (cefepime 69.0% vs. 70.3%, p = 0.658; cefuroxime 71.8% vs.72.4%, p = 0.843). None of the differences in rates of resistance were statistically significant except for piperacillin-tazobactam (p = 0.021) and levofloxacin (30.8 vs. 22.9, p = 0.006). (Table 6.2)

Table 6.2 Univariate analysis results of *S. aureus* and *K. pneumoniae* resistance to specific antibiotics by gender

S.aureus				K.pneumoniae			
Antibiotic	Males n=399(%)	Females n=328(%)	p-value	Antibiotic	Males n=525(%)	Females n=422(%)	p-value
Ampicillin	472 (93.7)	405(95.3)	0.278	Mezlocillin	430(81.9)	342(81.0)	0.734
Amoxiclav***	251 (49.8)	242(56.9)	0.030	Ampicillin/sulbactam	391(74.5)	322(76.3)	0.517
Oxacillin	247 (49.0)	241(56.7)	0.901	Amoxclav	330(62.9)	281(66.6)	0.233
Penicillin	471(93.5)	405(95.3)	0.019	Imipenem	18(3.4)	12(2.8)	0.609
Imipenem	252 (50.0)	244(57.4)	0.024	Meropenem	9(1.7)	3(0.7)	0.170
Meropenem	255 (50.6)	243(57.2)	0.045	Ertapenem	15(2.9)	11(2.6)	0.815
Ertapenem	253 (50.2)	243(57.2)	0.034	Cefazolin	377(71.8)	306(72.5)	0.811
Cefoxitin	260 (51.6)	190(44.7)	0.037	Ceftazidime	370(70.5)	292(69.2)	0.669
Cefepime	250 (49.6)	242(56.9)	0.026	Cefuroxime	380(72.4)	303(71.8)	0.843
Gentamicin	256 (50.8)	242(56.9)	0.061	Cefotaxime	368(70.1)	290(68.7)	0.648
Tobramycin	254(50.4)	248(58.4)	0.015	Cefoxitin	69(13.1)	57(13.5)	0.870
Clindamycin	186 (36.9)	183(43.1)	0.056	Cefepime	369(70.3)	291(69.0)	0.658
Erythromycin	230 (45.6)	217(51.1)	0.099	Piperacillin	412(78.5)	333(78.9)	0.871
Azithromycin	227 (45.0)	214(50.4)	0.106	Pip_Tazo*	144(27.4)	145(34.4)	0.021
Ciprofloxacin	232 (46.0)	211(49.7)	0.272	Aztreonam	370(70.5)	294(69.7)	0.787
Levofloxacin	223 (44.3)	185(43.5)	0.826	Gentamycin	328(62.5)	258(61.1)	0.673
Moxifloxacin	221 (43.9)	184(43.3)	0.865	Tobramycin	342(65.1)	283(67.1)	0.536
Trim-Sulfameth**	198(39.3)	190(44.8)	0.089	Amikacin	23(4.4)	20(4.7)	0.792
Tetracyclin	249(49.4)	233(54.8)	0.100	Ciprofloxacin	224(42.7)	203(48.1)	0.095
Vancomycin	6(1.2)	3(0.7)	0.453	Levofloxacin	120(22.9)	130(30.8)	0.006
Fosfomycin	3 (0.6)	1(0.2)	0.404	Moxifloxacin	241(45.9)	218(51.7)	0.078
Rifampin	83(16.5)	91(21.4)	0.228	Chlorampenicol	248(47.2)	209(49.5)	0.484
Fusidic Acid	7 (1.4)	4(0.9)	0.530	Trim-Sulfameth	373(71.1)	304(72.0)	0.737
Synercid	6(1.2)	4(0.9)	0.054	Tetracycline	240(45.7)	205(48.6)	0.380
Teicoplanin	2(0.4)	2(0.5)	0.864	Tigecycline	38(7.2)	40(9.5)	0.213
				Fosfomycin	42(8.0)	44(10.4)	0.196

* Piperacillin-Tazobactam; ** Trimethoprim-Sulfamethaxazole, ***Amoxicillin-Clavulanate

6.3.4 Patterns of antimicrobial resistance rate by province

The proportions of SA resistant isolates varied by geographical location and were consistently higher among isolates from Gauteng province as compared to Free State and the Western Cape provinces. For example, significant differences were found in the proportion of isolates resistant to the beta-lactams ((oxacillin 48.9% vs. 61.9% vs. 39.5%, $p = <0.001$) carbapenems (etrapenem (51.1% vs. 62.8 vs. 40.1%, $p = <0.001$), cephalosporins (cefepime 48.9% vs. 62.5% vs. 39.5%, $p = <0.001$)) and fluoroquinolone (ciprofloxacin 42.6% vs 60.7% vs 26.7%, $p = <0.001$), among others. There were statistically significant differences in the distribution of SA isolates resistant to most of the antibiotics tested between the three provinces except for fosfomycin, synercid, fusidic acid, and vancomycin, $p = >0.05$. There were only 4 isolates resistant to teicoplanin and only 9 isolates resistant to vancomycin, all were reported from Gauteng province. (Table 6.3) The proportions of KP resistant isolates were higher in the Free State province for a number of antibiotics except amoxicillin-clavulanate, fosfomycin, levofloxacin, piperacillin-tazobactam and tigercycline. There were statistically significant differences among three provinces (Free State, Gauteng and Western Cape respectively) in the proportion of KP isolates resistant to beta-lactams ((amoxycillin–clavulante (50.0% vs. 67.9% vs. 58.7%, $p = 0.001$); piperacillin-tazobactam (20.3% vs. 34.2% vs. 22.1%, $p = <0.001$)), and fluoroquinolones ((ciprofloxacin (50.0% vs. 47. 1% vs. 37.3%, $p = 0.015$); levofloxacin (25.0% vs. 28.8% vs. 18.5%, $p = 0.004$)) among others. (Table 6.3)

Table 6.3 Univariate analysis results of *S. aureus* and *K. pneumoniae* resistance to selected antibiotics by province

S. aureus					*K. pneumonia*				
Antibiotic	*FS n=47(%)	GA n=651(%)	WC n=334(%)	p-value	Antibiotic	FS n=64(%)	GA n=705(%)	WC n=276(%)	p-value
Ampicillin	42 (89.4)	623(95.7)	312(93.4)	0.081	Mezlocillin	56 (87.5)	583 (82.7)	220(79.7)	0.284
Amoxiclav	23 (48.9)	408(62.7)	132(39.5)	<0.001	Ampicillin/Sulbactam	51 (80.0)	539 (76.5)	202(73.2)	0.424
Oxacillin	23(48.9)	403(61.9)	132(39.5)	<0.001	Amoxclav	32 (50.0)	479 (67.9)	162(58.7)	0.001
Penicillin	42(89.4)	623(95.7)	311(93.1)	<0.001	Imipenem	3(4.7)	21(3.0)	12(4.4)	0.488
Imipenem	23(48.9)	409(62.8)	134(40.1)	<0.001	Meropenem	2(3.1)	8(1.1)	4(1.5)	0.408
Meropenem	23(48.9)	409(62.8)	136(40.7)	<0.001	Ertapenem	4 (6.3)	20 (2.8)	6(2.2)	0.212
Ertapenem	24 (51.1)	409(62.8)	134(40.1)	<0.001	Cefazolin	52 (81.3)	516 (73.2)	193(69.9)	0.173
Cefoxitin	25 (53.2)	256(39.3)	204(61.1)	<0.001	Ceftazidime	51(79.7)	503(71.4)	186(67.4)	0.129
Cefepime	23 (48.9)	407(62.5)	132(39.5)	<0.001	Cefuroxime	53 (82.8)	515 (73.1)	193(69.9)	0.110
Gentamicin	23(48.9)	417(64.1)	129(38.6)	0.225	Cefotaxime,	51(79.7)	500(70.9)	185(67.0)	0.120
Tobramycin	25(53.2)	419(64.4)	126(37.7)	<0.001	Cefoxitin	4 (6.3)	99 (14.0)	30(10.9)	0.112
Clindamycin	19 (40.4)	305(46.9)	102(30.5)	<0.001	Cefepime	52 (81.3)	501 (71.1)	185(67.0)	0.072
Erythromycin	22 (46.8)	376(57.8)	115(34.4)	<0.001	Piperracillin	55 (85.9)	565 (80.1)	209(75.7)	0.124
Azithromycin	20 (42.6)	373(57.3)	114(34.1)	<0.001	Pip-Tazo	13 (20.3)	241 (34.2)	61(22.1)	<0.001
Ciprofloxacin	20 (42.6)	395(60.7)	89(26.7)	<0.001	Aztreonam,	52(81.3)	502(71.2)	188(68.1)	0.111
Levofloxacin	16(34.0)	379(58.2)	74(22.2)	<0.001	Gentamycin	48 (75.0)	433 (61.4)	175(63.4)	0.096
Moxifloxacin	15(31.9)	374(57.5)	76(22.8)	<0.001	Tobramycin	45 (70.3)	483 (68.5)	168(60.9)	0.060
Trim-Sulfameth	13(27.7)	369(56.8)	70(21.0)	<0.001	Amikacin	5 (7.8)	36(5.1)	6(2.2)	0.057
Tetracyclin	15(31.9)	426(65.4)	108(32.3)	0.309	Ciprofloxacin	32 (50.0)	332 (47.1)	103(37.3)	0.015
Vancomycin	0(0.0)	9(1.4)	0(0.0)	0.070	Levofloxacin	16 (25.0)	203 (28.8)	51(18.5)	0.004
Fosfomycin	1(2.1)	2(0.3)	1(0.3)	0.100	Moxifloxacin	32 (50.0)	346 (49.1)	121(43.8)	0.314
Rifampicin	10(21.3)	137(21.0)	47(14.1)	0.065	Chloramphenicol	42 (65.6)	378 (53.6)	96(34.8)	<0.001
Fusidic acid	1(2.1)	9(1.4)	1(0.3)	0.145	Trim-Sulfameth	51 (79.7)	517 (73.3)	185(67.0)	0.053
Synercid	2(4.3)	10(1.5)	2(0.6)	0.027	Tetracycline	34 (53.1)	336 (47.7)	113(40.9)	0.086
Teicoplanin	0(0.0)	4(0.6)	0(0.0)	0.103	Tigecycline	2 (3.1)	55 (7.8)	26(9.4)	0.238
					Fosfomycin	4 (6.3)	74 (10.5)	14(5.1)	0.020

*FS 'Free State'; GA 'Gauteng'; WC 'Western Cape',

6.3.5 Patterns of antimicrobial resistance rate over time

Higher proportions of SA isolates reported between July and December in 2010 were resistant to various antibiotics compared with those reported between January and June in 2011. For oxacillin (57.1% vs. 51.1%, p = 0.052) and for vancomycin out of the 9 resistant isolates reported, 5 were from 2010 and 4 from 2011 (1.0% vs. 0.8%, p = 0.733). The observed variation in the proportions of SA resistance was not significantly different for most antibiotics except for amoxicillin-clavulanate (57.9% vs. 51.3%, p = 0.033); ertapenem (58.1% vs. 51.8%, p = 0.045) and cefepime (57.7% vs. 51.3%, p = 0.039).

For KP, a similar pattern of resistance was observed. However, the proportion of antibiotic resistance was higher in the 2011 period of the study compared to the 2010 period with some insignificant exceptions: amikacin, ertapenem and tigercycline showed slightly higher rates

of KP resistance during the first 6 months of the study compared with the last 6 months of the study. There were variations in the proportions of resistant isolates between the two 6 month's periods of July – December 2010 and January to June 2011. Although this variation was not significantly different for most antibiotics, significant differences were observed for the following antibiotics: ampicillin/salbactum (73.0% vs. 78.4%, p = 0.044), fosfomycin (10.6% vs. 7.2%, p = 0.050) and mezlocilin (79.4% vs. 84.8%, p = 0.023). (Table 6.4) The findings for SA are consistent with the winter respiratory infection season, and the findings for KP are typical for the summer gastrointestinal infection season in the southern hemisphere. Respiratory infections are known to be more common in winter. This corresponds to SA isolations which were more commonly isolated in 2010, representing the colder months of the year. Shedding of SA from the nasal carriage site is likely to be more common in winter due to respiratory infections. In contrast, diarrhoeal infections are more common in summer and KP commonly colonize the intestinal tract and is therefore more likely to cause bacteraemia associated with diarrhea during the summer months.

Table 6.4 Univariate analysis results of *S. aureus* and *K. pneumoniae* resistance of each antibiotic by year

| *S. aureus* | | | | *K. pneumoniae* | | | |
Antibiotic	2010 n=515 (%)	2011 n=517(%)	p-value	Antibiotic	2010 n=500(%)	2011 n=545(%)	p-value
Ampicillin	482 (93.6)	495(95.7)	0.124	Mezlocillin	397(79.4)	462(84.8)	0.023
Amoxiclav	298 (57.9)	265(51.3)	0.033	Ampicillin/Sulbactam	365(73.0)	427(78.4)	0.044
Oxacillin	294(57.1)	264(51.1)	0.052	Amoxiclav	311(62.2)	362(66.4)	0.154
Imipenem	297 (57.7)	269(52.0)	0.069	Imipenem	14(2.8)	22(4.0)	0.274
Meropenem	297 (57.7)	271(52.4)	0.090	Meropenem	10(2.0)	4(0.7)	0.075
Ertapenem	299 (58.1)	268(51.8)	0.045	Ertapenem	19(3.8)	11 (2.0)	0.085
Cefoxitin	228 (44.3)	257(49.7)	0.080	Cefazolin	357(71.4)	404(74.1)	0.322
Cefepime	297 (57.7)	265(51.3)	0.039	Ceftazidime	349(69.8)	391(71.7)	0.490
Gentamicin	293 (56.9)	276(53.4)	0.257	Cefuroxime	359(71.8)	402(73.8)	0.476
Tobramycin	292(56.7)	278(53.8)	0.344	Cefotaxime	348(69.6)	388(71.2)	0.573
Clindamycin	226 (43.9)	200(38.7)	0.090	Cefoxitin	62(12.4)	71(13.0)	0.761
Erythromycin	274 (53.2)	239(46.2)	0.025	Cefepime	349(69.8)	389(71.4)	0.576
Azithromycin	269 (52.2)	238(46.0)	0.046	Piperracillin	386(77.2)	443(81.3)	0.103
Ciprofloxacin	258 (50.1)	246(47.6)	0.419	Pip- Tazo*	154(30.8)	161(29.5)	0.658
Levofloxacin	238 (46.2)	231(44.7)	0.621	Aztreonam	350(70.0)	392(71.9)	0.493
Moxifloxacin	240(46.6)	225(43.5)	0.320	Gentamycin	307(61.4)	349(64.0)	0.378
Trim-Sulfameth	226(44.0)	226(43.7)	0.934	Tobramycin	328(65.6)	368(67.5)	0.510
Tetracyclin	272(52.8)	277(53.6)	0.806	Amikacin	27(5.4)	20(3.7)	0.178
Vancomycin	5(1.0)	4(0.8)	0.733	Ciprofloxacin	211(42.2)	256(47.0)	0.121
Fosfomycin	2 (0.4)	2(0.4)	0. 997	Levofloxacin	122(24.4)	148(27.2)	0.309
Rifampin	105(20.4)	89(17.2)	0.192	Moxifloxacin	226(45.2)	273(50.1)	0.114
Fusidic Acid	6 (1.2)	5(1.0)	0.757	Chlorampenicol	253(50.6)	263(48.3)	0.449
Synercid	10(1.9)	4(0.8)	0.105	Trim-Sulfameth	362(72.4)	391(71.7)	0.813
Teicoplanin	2(0.4)	2(0.4)	0.997	Tetracycline	230(46.0)	253(46.4)	0.891
				Tigecycline	40(8.0)	43(7.9)	0.948
				Fosfomycin	53(10.6)	39(7.2)	0.050

6.3.6 Age related distribution of patterns of *S aureus* resistance

The pattern of SA resistance by age was varied. From Table 6.5, the highest rates of resistance were in the under 5 year's age-group. Resistance to cefepime, a 4th generation cephalosporin in the under 5 years was >60%. Significant differences existed in the rates of resistance by the different age - groups (p < 0.001). The proportion of MRSA was 63.3% in the <5 year age-group, indicating that there were significant differences in the rates of resistance between the different age - groups (p = <0.001). There were 9 isolates resistant to vancomycin, 4 (4/324) isolates in the under 5 years age-group, a single isolate (1/91) in the 40 - 49 years age-group and the remaining 4 isolates had no data on age. Only a single isolate (1/324), resistant to teicoplanin was reported and was identified in the under 5 years age-group. For other antibiotics such as fusidic acid, 3 (3/324) resistant isolates were identified in

the under 5 year's age-group, 1(1/121) in the 30 - 39 years age-group, 1 (1/91) in the 40 - 49

year age-group and 1 (1/48) in the >70 years age group; for synercid there were 7 (7/324)

resistant isolates in the <5 years age-group, 1 (1/121) in the 30-39 years age-group and 1

(1/48) in the >70 years age group. Comparatively, the 20 - 29 years age-group had lower rates

of resistance amongst the different age-groups and there was no identified isolate resistant to

vancomycin, linezolid and fusidic acid.(Table 6.5)

Table 6.5 Univariate analysis results of *S. aureus* resistance to each antibiotic by age group

Antibiotic	Age group									p-value
	<5 n=324(%)	5-9 n=22(%)	10-19 n=31(%)	20-29 n=88(%)	30-39 n=121(%)	40-49 n=91(%)	50-59 n=82(%)	60-69 n=71(%)	>70 n=48(%)	
Amoxiclav	208(64.2)	7(31.8)	14(45.2)	32(36.4)	62(51.2)	46(50.6)	34(41.5)	26(36.6)	20(41.7)	<0.001
Ampicillin	315(97.2)	20(90.9)	28(90.3)	79(89.8)	114(94.2)	84(92.3)	77(93.9)	65(91.6)	44(91.7)	0.165
Azithromycin	202(62.4)	9(40.9)	14(45.2)	25(28.4)	49(40.5)	36(39.6)	30(36.6)	24(33.8)	19(39.6)	<0.001
Cefepime	208(64.2)	7(31.8)	14(45.2)	32(36.4)	60(49.6)	47(51.7)	34(41.5)	26(36.6)	20(41.7)	<0.001
Cefoxitin	124(38.3)	15(68.2)	17(54.8)	57(64.8)	61(50.4)	46(50.6)	48(58.5)	45(63.4)	28(58.3)	<0.001
Ciprofloxacin	166(51.2)	9(40.9)	10(32.3)	32(36.4)	52(43.0)	43(47.3)	35(42.7)	27(38.0)	22(45.8)	0.156
Clindamycin	163(50.3)	7(31.8)	13(41.9)	20(22.7)	45(37.2)	34(37.4)	27(32.9)	22(31.0)	16(33.3)	<0.001
Ertapenem	211(65.1)	8(36.4)	14(45.2)	32(36.4)	61(50.4)	46(50.6)	35(42.7)	26(36.6)	20(41.7)	<0.001
Erythromycin	201(62.0)	10(45.5)	14(45.2)	26(29.6)	49(40.5)	38(41.8)	30(36.6)	24(33.8)	20(41.7)	<0.001
Fosfomycin	2(0.6)	1(4.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0(0.0)	1(1.4)	0(0.0)	0.159
Fusidic Acid	3(0.9)	0 (0.0)	0 (0.0)	0 (0.0)	1(0.8)	1(1.1)	0 (0.0)	0 (0.0)	1(2.1)	0.861
Gentamicin	213(65.7)	12(54.6)	13(41.9)	36(40.9)	58(47.9)	46(50.6)	30(36.6)	20(28.2)	22(45.8)	<0.001
Imipenem	209(64.5)	8(36.4)	14(45.2)	32(36.4)	61(50.4)	46(50.6)	35(42.7)	26(36.6)	21(43.8)	<0.001
Levofloxacin	154(47.5)	7(31.8)	11(35.5)	27(30.7)	51(42.2)	41(45.1)	30(36.6)	25(35.2)	19(39.6)	0.114)
Meropenem	209(64.5)	9(40.9)	14(45.2)	32(36.4)	62(51.2)	46(50.6)	35(42.7)	27(38.0)	20(41.7)	<0.001
Moxifloxacin	154(47.5)	6(27.3)	12(38.7)	28(31.8)	50(41.3)	39(42.9)	30(36.6)	24(33.8)	19(39.6)	0.116
Oxacillin	205(63.3)	7(31.8)	14(45.2)	32(36.4)	60(49.6)	46(50.6)	34(41.5)	26(36.6)	20(41.7)	<0.001
Penicillin	315(97.2)	20(90.9)	28(90.3)	79(89.8)	114(94.2)	84(92.3)	77(93.9)	65(91.6)	43(89.6)	0.123
Rifampin	47(14.5)	6(27.3)	6(19.4)	17(19.3)	26(21.5)	27(29.7)	14(17.1)	8(11.3)	8(16.7)	0.047
Synercid	7(2.2)	0 (0.0)	0(0.0)	0 (0.0)	1(0.8)	0 (0.0)	0 (0.0)	0 (0.0)	1(2.1)	0.370
Teicoplanin	1(0.3)	0 (0.00)	0 (0.00)	0 (0.0)	0 (0.0)	0(0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.989
Tetracyclin	189(58.3)	9(40.9)	13(41.9)	40(45.5)	55(45.5)	45(49.5)	32(39.0)	25(35.2)	20(41.7)	0.003
Tobramycin	203(62.7)	12(54.6)	16(51.6)	37(42.1)	60(49.6)	46(50.6)	32(39.0)	26(36.6)	23(47.9)	<0.001
Sulfamethoxazole	160(49.5)	8(36.4)	8(25.8)	27(30.7)	51(42.2)	41(45.1)	22(26.8)	19(26.8)	12(25.0)	<0.001
Vancomycin	4(1.2)	0(0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1(1.1)	0 (0.0)	0 (0.0)	0(0.0)	0.628

6.3.7 Age related distribution of patterns of *K. pneumoniae* resistance

There was wide variation in KP resistance by age-group with significant differences observed

amongst the following antibiotics: cephalosporins (cefazolin, cefepime, cefotaxime,

ceftazidime); fluoroquinolones (ciprofloxacin, levofloxacin) (p < 0.05). Beta-lactams i.e.

amoxicillin-clavulanate, piperacillin-tazobactam and carbapenems (imipenem, etrapenem),

showed no significant differences in resistance rates between different age-groups (p≥0.05).

Even though differences in rates of antibiotic resistance were observed amongst some

aminoglycoside (amikacin, tobramycin), such differences were not statistically significant (p ≥ 0.05) except gentamicin (p = 0.007). The lowest number of resistant isolates was from the 5 - 9 years age-group with no resistant isolate identified to levofloxacin and tigercycline reported in this age-group. Higher rates of KP resistant isolates were in the < 5 years age-group. Resistance to cefotaxime ranged from 57.6% - 81.8%, (p = 0.003), showing high rates of ESBL presence across all age-groups. (Table 6.6)

Table 6.6 Univariate analysis results of *K. pneumoniae* resistance to each antibiotic by age group

Antibiotic	Age group									p-value
	<5 n=340(%)	5-9 n=11(%)	10-19 n=33(%)	20-29 n=80(%)	30-39 n=112(%)	40-49 n=92(%)	50-59 n=118(%)	60-69 n=88(%)	>=70 n=59(%)	
Amikacin	16(4.7)	1(9.1)	1(3.0)	3(3.8)	6(5.4)	2(2.2)	4(3.4)	7(8.0)	2(3.4)	0.742
Amoxiclav	230(67.7)	7(63.6)	22(66.7)	54(67.5)	75(67.0)	51(55.4)	75(63.6)	54(61.4)	32(54.2)	0.381
Ampicillin/Sulbactam	279(82.1)	8(72.7)	27(81.8)	59(73.8)	87(77.7)	66(71.7)	84(71.2)	65(73.9)	39(66.1)	0.088
Aztreonam	269(79.1)	8(72.7)	26(78.8)	51(63.8)	82(73.2)	60(65.2	78(66.1)	57(64.8)	34(57.6)	0.002
Cefazolin	273(80.3)	8(72.7)	27(81.8)	55(68.8)	82(73.2)	62(67.4)	80(67.8)	60(68.2)	37(62.7)	0.017
Cefepime	267(78.5)	8(72.7)	26(78.8)	51(63.8)	81(72.3)	60(65.2)	76(64.4)	58(65.9)	34(57.6)	0.004
Cefotaxime	266(78.2)	8(72.7)	27(81.8)	51(63.8)	81(72.3)	60(65.2)	76(64.4)	57(64.8)	34(57.6)	0.003
Cefoxitin	24(7.1)	1(9.1)	5(15.2)	13(16.3)	19(17.0)	10(10.9)	22(18.6)	17(19.3)	6(10.2)	0.007
Ceftazidime	267(78.5)	8(72.7)	27(81.8)	52(65.0)	82(73.2)	60(65.2)	76(64.4)	57(64.8)	34(57.6)	0.003
Cefuroxime	272(80.0)	8(72.7)	28(84.9)	53(66.3)	84(75.0)	63(68.5)	77(65.3)	61(69.3)	36(61.0)	0.004
Chlorampenicol	152(44.7)	3(27.3)	21(63.6)	43(53.8)	59(52.7)	48(52.2)	57(48.3)	53(60.2)	25(42.4)	0.069
Ciprofloxacin	119(35.0)	4(36.4)	18(54.6)	36(45.0)	63(56.3)	40(43.5)	62(52.5)	51(58.0)	30(50.9)	<0.001
Ertapenem	9(2.7)	1(9.1)	0(0.0)	3(3.8)	1(0.9)	1(1.1)	5(4.2)	6(6.8)	1(1.7)	0.184
Fosfomycin	28(8.2)	1(9.1)	3(9.1)	2(2.5)	7(6.3)	5(5.4)	15(12.7)	6(6.8)	7(11.9)	0.289
Gentamicin	244(71.8)	7(63.6)	23(69.7)	44(55.0)	70(62.5)	54(58.7)	66(55.9)	54(61.4)	30(50.9)	0.007
Imipenem	14(4.1)	1(9.1)	2(6.1)	2(2.5)	2(1.8)	3(3.3)	2(1.7)	5(5.7)	2(3.4)	0.693
Levofloxacin	42(12.4)	0(0.0)	12(36.4)	21(26.3)	45(40.2)	28(30.4)	43(36.4)	36(40.9)	20(33.9)	<0.001
Mezlocillin	297(87.4)	9(81.8)	31(93.9)	64(80.0)	92(82.1)	72(78.3)	91(77.1)	66(75.0)	43(72.9)	0.015
Meropenem	6(1.8)	1(9.1)	0(0.0)	0(0.0)	0(0.0)	1(1.1)	2(1.7)	3(3.4)	0(0.0)	0.158
Moxifloxacin	132(38.8)	4(36.4)	20(60.6)	39(48.8)	64(57.1)	42(45.7)	68(57.6)	50(56.8)	32(54.2)	0.001
Piperracillin	290(85.3)	8(72.7)	31(93.9)	63(78.8)	89(79.5)	68(73.9)	89(75.4)	65(73.9)	41(69.5)	0.010
Pip-tazobactam	95(27.9)	4(36.4)	10(30.3)	25(31.3)	38(33.9)	25(27.2)	40(33.9)	32(36.4)	20(33.9)	0.799
Tetracycline	149(43.8)	4(36.4)	19(57.6)	32(40.0)	56(50.0)	42(45.7)	56(47.5)	52(59.1)	27(45.8)	0.225
Tigecycline	26(7.7)	0(0.0)	5(15.2)	10(12.5)	10(8.9)	6(6.5)	9(7.6)	8(9.1)	2(3.4)	0.471
Tobramycin	247(72.7)	7(63.6)	22(66.7)	49(61.3)	78(69.6)	56(60.9)	71(60.2)	57(64.8)	33(55.9)	0.087
Trim-sulfam	258(75.9)	8(72.7)	27(81.8)	60(75.0)	82(73.2)	68(73.9)	83(70.3)	63(71.6)	31(52.5)	0.043

6.3.8 Hospital related distribution of patterns of S.aureus resistance

The rate of SA resistance across hospitals was widely varied, showing significant differences amongst most antibiotics such as amoxicillin-clavulanate, oxacillin, imipenem, meropenem, cefepime among others (p ≤ 0.001). The rates of SA resistance to oxacillin, a marker for

MRSA, was significantly different across the different hospitals; ranging from 36.3% - 83.2% ($p \leq 0.001$) and highest rates being (243/292, 83.2%) at CHB hospital. Out of the 9 vancomycin resistant isolates, 4 (1.4%) were from CHB, 1 (1.1%) was from CMJAH and 4 (2.5%) were from SBPAH. For teicoplanin resistant isolates, 1 (1.1%) was from CMJAH, 1 (0.3%) from CHB and 2 (1.3%) were from SBPAH. As for fusidic acid, 3 (1.0%) isolates were from CHB, 2 (2.3%) were from CMJAH, 1 (0.5%) was from GSH, 1 (2.1%) from UH and 4(2.5%) were from SBPAH. There were no resistant isolates to linezolid and daptomycin from any of the hospitals and rates of MRSA were generally lower at SBPAH compared to all other hospitals. (Table 6.7)

Table 6.7 Univariate analysis of *S. aureus* resistance to each antibiotic by hospital

Antibiotic	CMJAH n=88(%)	CHB n=292(%)	GSH n=215(%)	HJH n=111(%)	SBPAH n=160(%)	TH n=119(%)	UH n=47(%)	p-value
Ampicillin	85 (96.6)	286 (98.0)	201(93.5)	104(93.7)	148 (92.5)	111(93.3)	42(89.4)	0.057
Amoxiclav	54 (61.4)	244 (83.6)	77(35.8)	49(44.1)	61(38.1)	55(46.2)	23(48.9)	<0.001
Oxacillin	53 (60.2)	243 (83.2)	77(35.8)	49 (44.1)	58(36.3)	55(46.2)	23(48.9)	<0.001
Imipenem	54(61.4)	244(83.6)	79(36.7)	50(45.1)	61(38.1)	55(46.2)	23(48.9)	<0.001
Meropenem	55(62.5)	244 (83.6)	79(36.7)	50 (45.1)	60 (37.5)	57(47.9)	23(48.9)	<0.001
Ertapenem	54 (61.4)	244 (83.6)	79(36.7)	50 (45.1)	61 (38.1)	55(46.2)	24(51.1)	<0.001
Cefoxitin	37(42.1)	54 (18.5)	139(64.7)	62(55.9)	103 (64.4)	65(54.6)	25(53.2)	<0.001
Cefepime	54 (61.4)	244 (83.6)	77(35.8)	49 (44.1)	60 (37.5)	55(46.2)	23(48.9)	<0.001
Gentamicin	52(59.1)	250(85.6)	72(33.5)	51(46.0)	64 40.0)	57(47.9)	23(48.9)	<0.001
Tobramycin	54(61.4)	245 (83.9)	70(32.6)	51 (46.0)	69 (43.1)	56(47.1)	25(53.2)	<0.001
Ciprofloxacin	52 (59.1)	236 (80.8)	48(22.3)	49 (44.1)	58 (36.3)	41(34.5)	20(42.6)	<0.001
Levofloxacin	49 (55.7)	227 (77.7)	37(17.2)	45 (40.5)	58 (36.3)	37(31.1)	16(34.0)	<0.001
Moxifloxacin	48 (54.6)	225 (77.1)	38(17.7)	44 (39.6)	57 (35.6)	38(31.9)	15(31.9)	<0.001
Clindamycin	47 (53.4)	167 (57.2)	61(28.4)	38 (34.2)	53 (33.1)	41(34.5)	19(40.4)	<0.001
Erythromycin	50 (56.8)	221 (75.7)	68(31.6)	45 (40.5)	60 (37.5)	47(39.5)	22(46.8)	<0.001
Azithromycin	50 (56.8)	218 (74.7)	66(30.7)	45(40.5)	60 (37.5)	48(40.3)	20(42.6)	<0.001
Trim-Sulfameth	50(56.8)	227(77.7)	37(17.2)	46(41.4)	46(28.9)	33(27.7)	13(27.7)	<0.001
Tetracyclin	54(61.4)	249 (85.3)	62(28.8)	59 (53.2)	64 (40.0)	46(38.7)	15(31.9)	<0.001
Vancomycin	1(1.1)	4(1.4)	0(0.0)	0(0.0)	4(2.5)	0(0.0)	0(0.0)	0.119
Rifampin	22 (25.0)	65 (22.3)	35(16.3)	27 (24.3)	23(14.4)	12(10.1)	10 (21.3)	0.015
Synercid	3 (3.4)	4 (1.4)	1(0.5)	0 (0.0)	3 (1.9)	1(0.8)	2(4.3)	0.169
Fosfomycin	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.9)	0 (0.0)	1(0.8)	1 (2.1)	0.331
Fusidic Acid	2 (2.3)	3 (1.0)	1(0.5)	0 (0.0)	4 (2.5)	0(0.0)	1(2.1)	0.234
Teicoplanin	1 (1.1)	1 (0.3)	0(0.0)	0(0.0)	2 (1.3)	0(0.0)	0(0.0)	0.392

6.3.9 Hospital related distribution of patterns of *K. pneumoniae* resistance

The pattern of KP resistance for most antibiotics was not significantly different across different hospitals except for amikacin, cefotaxime, cefuroxime, cefepime, tobramycin and

piperacillin-tazobactam (p < 0.05). There was no KP resistant isolate reported from TH against ertapenem and only 1 resistant isolate to ertapenem was reported from CMJAH. A total of 36 isolates representing 3.4% (36/1045) were resistant to imipenem, and the range of resistance across hospitals was 2.0% - 8.1%, but the variation not statistically significant (p = 0.236). KP resistance to piperacilin/tazobactam across all hospitals ranged from 19.3% - 35.5% (p = 0.005) showing a statistically significant variation in rates of resistance across different hospitals. The proportion of ESBL-KP was lowest at SBPAH and highest at UH (61.4 - 79.7%, P = 0.012). It is evident that SBPAH generally had lower rates of ESBL-KP producing isolates compared to other hospitals. (Table 6.8)

Table 6.8 Results of univariate analysis of *K. pneumoniae* resistance to each antibiotic by hospital

Antibiotic	CMJAH n=97(%)	CHB n=349(%)	GSH n=166(%)	HJH n=62(%)	SBPAH n=197(%)	TH n=110(%)	UH n=64(%)	p-value
Mezlocillin	81(83.5)	298(85.4)	133(80.1)	52(83.9)	152(77.2)	87(79.1)	56(87.5)	0.202
Amp-Sulb	78(80.4)	272(77.9)	120(72.3)	50(80.7)	139(70.6)	82(74.6)	51(79.7)	0.279
Amoxiclav	65(67.0)	244 (69.9)	102(61.5)	45(72.6)	125(63.5)	60(54.6)	32(50.0)	0.007
Imipenem	4(4.1)	7(2.0)	8(4.8)	5(8.1)	5(2.5)	4(3.6)	3(4.7)	0.236
Meropenem	1(1.0)	3(0.9)	4(2.4)	2(3.2)	2(1.0)	0(0.0)	2(3.1)	0.323
Ertapenem	1(1.0)	9(2.6)	6(3.6)	3(4.8)	7(3.6)	0(0.0)	4(6.3)	0.193
Cefazolin	74(76.3)	265(75.9)	119(71.7)	47(75.8)	130(66.0)	74(67.3)	52(81.3)	0.079
Ceftazidime	72(74.2)	261(74.8)	113(68.1)	47(75.8)	123(62.4)	73(66.4)	51(79.7)	0.022
Cefuroxime	72(74.2)	268(76.8)	117(70.5)	48(77.4)	127(64.5)	76(69.1)	53(82.8)	0.021
Cefoxitin	10(10.3)	54(15.5)	21(12.7)	10(16.1)	25(12.7)	9(8.2)	4(6.3)	0.239
Cefotaxime	72(74.2)	260(74.5)	113(68.1)	47(75.8)	121(61.4)	72(65.5)	51(79.7)	0.012
Cefepime	72(74.2)	261(74.8)	113(68.1)	46(74.2)	122(61.9)	72(65.5)	52(81.3)	0.011
Piperracillin	81(83.5)	286(82.0)	124(74.7)	51(82.3)	147(74.6)	85(77.3)	55(85.9)	0.141
Pip-Tazo*	34(35.1)	116(33.2)	32(19.3)	22(35.5)	69(35.0)	29(26.4)	13(20.3)	0.005
Aztreonam	72(74.2)	261(74.8)	115(69.3)	46(74.2)	123(62.4)	73(66.4)	52(81.3)	0.021
Gentamicin	60(61.9)	223(63.9)	108(65.1)	43(69.4)	107(54.3)	67(60.9)	48(75.0)	0.061
Tobramycin	66(68.0)	253(72.5)	105(63.3)	45(72.6)	119(60.4)	63(57.3)	45(70.3)	0. 017
Amikacin	2(2.1)	14(4.0)	6(3.6)	2(3.2)	18(9.1)	0(0.0)	5(7.8)	0.005
Ciprofloxacin	40(41.2)	162(46.4)	68(41.0)	37(59.7)	93(47.2)	35(31.8)	32(50.0)	0.013
Levofloxacin	21(21.7)	99(28.4)	32(19.3)	25(40.3)	58(29.4)	19(17.3)	16(25.0)	0.006
Moxifloxacin	46(47.4)	170(48.7)	80(48.2)	35(56.5)	95(48.2)	41(37.3)	32(50.0)	0.319
Chlorampenicol	57(58.8)	193(55.3)	54(32.5)	37(60.0)	91(46.2)	42(38.2)	42(65.6)	<0.001
Trim-Sulfameth	71(73.2)	257(73.6)	111(66.9)	49(79.0)	140(71.1)	74(67.3)	51(79.7)	0.284
Tetracyclin	41(42.3)	162(46.4)	72(43.4)	29(46.8)	104(52.8)	41(37.3)	34(53.1)	0.154
Tigecycline	12(12.4)	27(7.7)	17(10.2)	3(4.8)	13(6.6)	9(8.2)	2(3.1)	0.306
Fosfomycin	8(8.3)	37(10.6)	8(4.8)	6(9.7)	23(11.7)	6(5.5)	4(6.3)	0.181

6.3.10 Analysis of factors associated with methicillin resistance to *S. aureus*

In the analysis of factors associated with MRSA, males with SA bacteraemia were significantly less likely to have MRSA (adjusted odds ratio (AOR) 0.63, confidence interval (CI) 0.46 - 0.86, p = 0.003) compared with females. Among children of <5 years, those above the age of 5 years were significantly less likely to have MRSA; this was true for children of 5 - 9 years and adults 20 - 29 years and ≥60 years. Using CMJAH as a reference, SA isolates from other hospitals, except CHB, were less likely to be methicillin resistant. Findings showing statistically significant less likelihood of MRSA isolates were observed from the GHS, HJ, and SBPAH, while SA isolates from CHB were nearly 3 times more likely to be methicillin resistant (AOR 2.91, CI 1.40 - 6.06, p = 0.004). (Table 6.9)

Table 6.9 Risk factors associated with methicillin-resistant *S. aureus* (MRSA) blood stream infections

		Univariate Analysis		Multivariate Analysis	
Characteristic	Positive MRSA (%)	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Sex					
Female	231/425(54)	1		1	
Male	231/504(45)	0.71(0.55-0.92)	0.010	0.63(0.46-0.86)	0.003
Age					
<5	192/324(59)	1		1	
5-9	6/22(27)	0.26(0.10-0.68)	0.006	0.23(0.08-0.72)	0.011
10-19	13/31(42)	0.50(0.24-1.05)	0.066	0.76(0.34-1.71)	0.506
20-29	30/88(34)	0.36(0.22-0.58)	<0.0001	0.40(0.23-0.71)	0.002
30-39	58/121(48)	0.63(0.42-1.07)	0.033	0.63(0.39-1.02)	0.062
40-49	45/91(49)	0.67(0.42-1.07)	0.096	0.92(0.55-1.55)	0.766
50-59	32/82(39)	0.44(0.27-0.72)	0.001	0.61(0.35-1.04)	0.070
60-69	24/71(34)	0.35(0.20-0.60)	<0.0001	0.40(0.22-0.73)	0.003
>70		0.45(0.24-0.84)	0.012	0.43(0.21-0.89)	0.023
Hospital					
Charlotte Maxeke Academic	47/88(53)	1		1	
Chris Hani Baragwanath	235/292(80)	3.60(2.16-5.98)	<0.0001	2.91(1.40-6.06)	0.004
GSH	70/215(33)	0.42(0.25-0.70)	0.001	0.32(0.16-0.63)	0.001
Helen Joseph	48/111(43)	0.66(0.38-1.17)	0.155	0.41(0.20-0.84)	0.016
Steve Biko Academic	55/160(34)	0.46(0.27-0.78)	0.004	0.32(0.16-0.65)	0.002
Tygerberg	52/119(44)	0.68(0.39-1.18)	0.167	0.55(0.27-1.12)	0.097
Universitas	22/47(47)	0.77(0.38-1.56)	0.465	0.89(0.36-2.15)	0.788
Province					
Free State	22/47(47)	1		-	-
Gauteng	385/651(59)	1.64(0.91-2.98)	0.101	-	-
Western Cape	122/334(37)	0.65(0.35-1.21)	0.176	-	-
Year					
2010	278/515(54)	1		1	
2011	251/517(49)	0.80(0.63-1.03)	0.081	0.79(0.58-1.08)	0.139

6.3.11 Analysis of factors associated with ESBL *K. pneumoniae*

Among KP isolates, there was no significant association observed between gender and ESBL-KP. Except for age-group less than 20 years, KP isolates from patients of age ≥20 years were significantly less likely to be ESBL producers. The analysis showed an inverse relationship, the higher the age the less the likelihood of ESBL production, e.g. patients >70 years were less likely to have ESBL-KP isolates (AOR 0.31, CI 0.17 − 0.57, p = <0.0001).

In multivariate models, with CMJAH as the reference group, KP isolates from SBPAH were significantly less likely to be ESBL producers (AOR 0.49, CI 0.25 − 0.95, p = 0.036). The same pattern showing lower odds of ESBL-KP was observed from the other hospitals (CHB, GSH, HJ and UH). However, the association was not statistically significant. (Table 6.10)

Table 6.10 Factors associated with extended spectrum beta-lactames (ESBLs) *K. pneumoniae*

Characteristic	Positive ESBL (%)	Univariate Analysis Odds Ratio (95% CI)	p-value	Multivariate Analysis Odds Ratio (95% CI)	p-value
Sex					
Female	273/422(65)	1		1	
Male	354/525(67)	0.89(0.68-1.16)	0.376	0.85(0.63-1.14)	0.282
Age					
<5	259/340(76)	1		1	
5-9	8/11(73)	0.83(0.22-3.22)	0.792	0.61(0.15-2.47)	0.491
10-19	25/33(76)	0.98(0.42-2.25)	0.957	0.92(0.37-2.26)	0.854
20-29	50/80(63)	0.52(0.31-0.87)	0.013	0.45(0.26-0.78)	0.005
30-39	75/112(67)	0.63(0.40-1.01)	0.055	0.60(0.37-0.98)	0.042
40-49	60/92(65)	0.59(0.36-0.96)	0.035	0.44(0.26-0.74)	0.002
50-59	72/118(61)	0.49(0.31-0.76)	0.002	0.42(0.26-0.68)	<0.0001
60-69	51/88(58)	0.43(0.26-0.70)	0.001	0.36(0.21-0.61)	<0.0001
>70	32/59(54)	0.37(0.21-0.66)	0.001	0.31(0.17-0.57)	<0.0001
Hospital					
Charlotte Maxeke Academic	69/97(71)	1		1	
Chris Hani Baragwanath	241/349(69)	0.91(0.55-1.48)	0.694	0.75(0.38-1.45)	0.389
GSH	111/166(67)	0.82(0.47-1.41)	0.473	0.60(0.30-1.19)	0.141
Helen Joseph	42/62(68)	0.85(0.43-1.70)	0.650	0.64(0.28-1.46)	0.288
Steve Biko Academic	116/197(59)	0.58(0.34-0.98)	0.042	0.49(0.25-0.95)	0.036
Tygerberg	72/110(65)	0.77(0.43-1.39)	0.382	0.50(0.24-1.04)	0.064
Universitas	50/64(78)	1.45(0.69-3.03)	0.324	0.96(0.38-2.44)	0.930
Province					
Free State	50/64(78)	1		-	-
Gauteng	468/705(66)	0.55(0.30-1.02)	0.058	-	-
Western Cape	183/276(66)	0.55(0.29-1.05)	0.069	-	-
Year					
2010	329/500(66)	1		1	-
2011	372/545(68)	1.12(0.86-1.45)	0.399	1.17(0.87-1.58)	0.308

6.3.12 Analysis of S. aureus/ MRSA, and K. pneumoniae/ESBL from three Johannesburg hospitals

In order to consider the possible role of race, which will also reflect socio-economic status, bacteraemic episodes and isolation from blood of *S. aureus* and MRSA; as well as *K. pneumoniae* and ESBL from CHB (predominantly black patients), CMJAH (all races, mainly white patients) and Helen Joseph Hospital (mainly white and coloured patients) were compared in Table 6.11. The proportion of MRSA from SA isolates was much higher at CHB (80%) representing predominantly black patients with relatively low socio-economic status, compared with the other two Johannesburg hospitals (proportion of MRSA isolates varying from 43% to 53%). The latter two hospitals accommodate mixed race patients with a small proportion of black patients originally coming from a higher socio-economic group than those at CHB. No obvious difference in the proportion of ESBL in KP among patients from the three hospitals was noted (proportions varied from 62% to 72%). The proportion of MRSA was significantly higher in females (54% vs 45%) however the proportions of ESBL in KP were very similar in females and in males (65% vs 67%). (Table 6.11)

Table 6.11 Bacteraemia isolates of *S. aureus*/ MRSA, and *K. pneumoniae*/ESBL from three Johannesburg hospitals representing different racial/ socio-economic populations

	Number of bacteraemic patients at three hospitals			
Hospitals	*S.aureus*	**MRSA (%)**	**K.*pneumoniae*	**ESBL (%)**
All	929	462 (49.7)	947	627 (66.2)
CHB	292	235 (80)	349	241 (69)
CHJAH	88	47 (53)	97	69 (71)
HJ	111	48 (43)	62	42 (68)

6.4 Discussion

This study aimed to provide data on the burden of AMR in South Africa using a sentinel surveillance system of collecting data on SA and KP blood culture isolates that have undergone susceptibility testing of all clinically relevant antibiotics with strict quality control measures. Drug susceptibility testing was carried out in a single (reference) laboratory. The purpose of doing this work was to validate the previous LIS/CDW approach and add to the understanding of the epidemiology of antimicrobial resistance in hospitals and provide a platform for policy change regarding antimicrobial use, regular surveillance of resistance patterns and hospital infection control.

6.4.1 Distribution of *S. aureus* and *K. pneumoniae* isolates

There were more isolates of SA and KP in the <5 years age-group, and more KP isolates in the 50 - 59 years age-group. It is not uncommon to find a high prevalence of bacteremia in children less than five years of age. However, among the 50 - 59 years age-group, it is assumed that the reason for high proportion of bacteremia, is likely to be a result of increasing colonization of KP in the communities. More isolates were reported from Gauteng province since more hospitals from this province were included in the study, and they were the largest hospitals and the volume of blood cultures from these 4 Gauteng hospitals might be higher than for the Western Cape and Free State provinces. Bias towards Gauteng sampling poses a major confounder when trends of resistance in provinces are considered. Rates of resistance observed from this province should therefore have been assessed separately. Despite this over representation there was insignificant variation in rates of resistance across hospitals. (Table 6.8)

Although it is still unclear why there is such variation in the type of isolates identified at HJH hospital and CMJAH, a possible reason might be the differences in infection control policies or dynamics of patient population i.e. differences in socioeconomic status. These two hospitals support patients from traditionally different population groups (HJH predominantly served patients from the south west of Johannesburg while CMJAH patients were mostly patients from the northern side of Johannesburg, meaning the overall prevalence of SA and KP might be different in the two geographical areas. More SA and KP isolates were reported among males compared to females showing preponderance for such infections among the male population. Again, the reason for the difference is unclear but, it might be a reflection of differences in risk exposures between the two populations or declining immune function in the male population presumably, due to higher prevalence of HIV infection, making them vulnerable to opportunistic bacteraemic episodes. HIV infection, especially when associated with low CD4 cell counts renders patients susceptible to infection, including invasive disease caused by SA and KP. (153, 154) The age distribution of MRSA is interesting and the proportions were highest in infants and children <5 years of age (59%), and in the 30-50 year age group (48-49%; see table 6.9), coinciding with HIV/AIDS prevalence. With regard to KP, the proportions of ESBLs were highest in the <20 years age groups (73%-76%; see table 6.10). The proportions of ESBL in the age groups 30 years to >70 years varied from 58-67%.

6.4.2 Antimicrobial resistance pattern of SA and KP isolates

The pattern of SA and KP resistance showed high proportion of resistant isolates to most of the conventional antibiotics, with β-lactam resistance of over 40% and MRSA crude rate of over 50%. This finding is consistent with previous studies that showed similar high rates of antibiotic resistance. (155, 156)

Resistance rates of SA to fusidic acid, synercid, teicoplanin and vancomycin were less than 2%. No resistant isolates were identified to linezolid and daptomycin. Despite these low prevalence rates, ongoing surveillance of drug resistance to this group of anti-microbial agents is important, as an increasing trend in resistance could have significant consequences for patient management. (157, 158) Earlier studies have shown that antimicrobial resistance to reserved antibiotics such as vancomycin has slowly been increasing (159) and this is a worrying development considering that antimicrobial-resistant strains acquired in hospital, could be transmitted nosocomially and eventually spread to the community. (160)

6.4.3 Patterns of antimicrobial resistance rate by gender

Even though minor differences were seen in gender prevalence of MRSA, these were not statistical significant. The same was the case with fluoroquinolone resistance in SA isolates. In contrast, for KP, significantly more cases of fluoroquinolone resistance occurred in females. It is unlikely that this could be attributed to the use of fluoroquinolones for the treatment of sexually transmitted infections (STIs) such as gonococcal infections. As a result of the emergence of drug resistance, such infections are no longer treated with this group of agents and historically these infections, although treated with fluoroquinolones, were probably more commonly diagnosed in males than in females. (161-163) The preponderance of fluoroquinolone resistance among KP isolates in females is likely to be related to the selective pressure of fluoroquinolone treatment of Gram-negative urinary tract infections which are very common and much more frequently encountered in females. (164)

6.4.4 Patterns of antimicrobial resistance rate by province

Four of the seven academic hospitals included in this study are in Gauteng Province, two in the Western Cape and one in the Free State. SA resistance rates to the various antimicrobial agents showed considerable variation e.g., resistance to β-lactams such as amoxicillin/clavulanate in the 4 Gauteng Province hospitals varied from 38.1% (SBPAH) to 83.6% (CHB) with a mean resistance rate of 60.9%, while the figures for the Western Cape Province were 35.8% for GSH and 46.2% for TH and a mean of 41.0%. The Free State (UH) figure was 48.9%. Because of the wide variation in resistance in hospitals to most of the antibiotics featuring in this study, it would be more meaningful for future studies to only compare the frequency of resistance between the hospitals rather than by province.

In a study by Bamford et al. performed on bacteraemic isolates from 7 academic hospitals, six of which featured in this present study, susceptibility of both SA and KP to selected antimicrobial agents was determined and analysed. Comparing the 2010 findings of Bamford et al. with those of this study, the respective resistance figures for SA are oxacillin (MRSA): 45% vs. 54.1% and erythromycin: 46% vs. 49.7%. In the case of KP, the respective resistance figures are 19% vs. 4.5% for amikacin; 57% vs. 62.8% for gentamicin and 37% vs. 44.7% for ciprofloxacin (123)

The proportions of KP resistant isolates to various antibiotics were higher in the Free State compared to Gauteng and Western Cape Provinces. We found that significant differences existed in the proportion of KP isolates resistant to antibiotics such as amoxicillin-clavulanate (50.0% vs. 67.9% vs. 58.7%, p = 0.001) and piperacillin-tazobactam (20.3% vs.34.2% vs. 22.1%, p = <0.001). The resistance rates are comparable to what is previously known about

KP resistance. (165-167) It is not immediately apparent why such geographical difference exists but it again may be attributed to differences in population dynamics, in these different provinces. However, this finding warrants further investigation to understand the dynamics of the observed differences including exploring differences in antibiotic prescribing policies or hospital infection control programmes in these provinces.

6.4.5 Patterns of antimicrobial resistance rate by year

The pattern of SA resistance differed between July to December 2010 and January to June 2011 with the 6 months period in 2010 showing higher proportions of resistant isolates than the 6 months period in 2011. It should be noted that in both years, data were gathered for half of the year (July 2010 to June 2011) as the Antimicrobial Resistance Surveillance and Research (ARSR) only started operating in July 2010. Caution should, therefore, be exercised in interpreting these findings as we would not know if this would have been different if data were to be collected for each complete calendar year.

The variation in resistance of SA to antibiotics between the two years was wide and statistically significant for: amoxicillin-clavulanate, cefepime, ertapenem etc. This finding is similar to that of a previous study that reported on retrospective data of SA and KP over a 5 year period. (168) For KP, we observed no significant differences in the variation of antibiotic resistance between the two time periods, for most of the antibiotics. The pattern favours an upward direction of resistance development, with higher rates of resistance in 2011. However, 6 months data may not be robust enough to unravel such differences. (31, 123, 165, 169)

6.4.6 Age related distribution of patterns of *S. aureus* resistance

The pattern of SA resistance shown in this study is consistent with previous findings. Resistance was shown to be higher in the < 5 years age-group. (168) High levels of resistance to 4th generation cephalosporins and MRSA rates of greater than 60% in this age-group poses a major clinical and public health challenge and is a worrisome development. (170) Vancomycin resistant SA isolates have been reported in previous studies done in Canada, UK and Japan.(115, 159, 171) Increasing magnitude of vancomycin resistant SA is of concern. (172) In our study, 9 vancomycin resistant isolates were found within this period.

Development of glycopeptides resistance poses major challenges as this is still an antibiotic of choice for MRSA.(173, 174) Lower rates of resistance for most of the antibiotics were observed in the 5 - 9 years age group than across the other different age-groups, with no resistant isolates reported to vancomycin, synercid and fusidic Acid. A possible explanation for this observation is probably a lower exposure to antibiotics amongst this age-group.

6.4.7 Age related distribution of patterns of *K. pneumoniae* resistance

The study showed that the largest number of ESBL-KP isolates (266/340, 78.2%) were in the under 5 years age-group, with significant differences between the different age-groups, (p=0.003). The lowest number of ESBL-KP isolates was in the 5 - 9 years age-group. No resistant isolates to levofloxacin and tigercycline were reported in this age-group. KP resistance to cefepime, a 4th generation cephalosporin, ranged from 56.7% to 78.8% (p=0.004) across all age-groups showing high resistance to extended spectrum cephalosporins. This study shows that antibiotic resistance in KP bacteraemia was high in the

younger age-group as opposed to the adult patients and this has been well documented in previous studies.

6.4.8 Hospital related distribution of patterns of *S. aureus* resistance

In a study by Bamford et al, susceptibility of both SA and KP to selected antimicrobial agents was determined and showed resistance rates that were comparable, with minimal degree of variation.(123) While the rates of SA resistance across hospitals varied widely, MRSA significantly differed across the hospitals (p=<0.001) and being highest at CHB hospital, similar to the finding that was previously reported. (123) This might be because of differences in infection prevention practices or prescribing policies between the different hospitals. The finding of high MRSA rates at CHB hospital is consistent with previous findings. (31) Of the 9 vancomycin resistant isolates, 4 were from CHB and 4 from SBPAH. This could be due to inadequate infection prevention measures at these hospitals. This is of concern as there is a danger of community spread of the resistant strain in the catchment area served by these hospitals.

6.4.9 Hospital related distribution of patterns of *K. pneumoniae* resistance

The study demonstrated a significant variation in the distribution of KP resistance across the different hospitals. The findings of this study are consistent with previous findings showing growing rates of resistance to the carbapenems ranging from 1% - 6.7%.(31, 123, 169) No KP isolate resistant to etrapenem and meropenem was reported from TH and only 1 resistant isolate was reported from CMJAH. It appears that none or lower number of isolates resistant to carbapenems were reported from HJH and UH. This could be due to low exposure of carbapenems as a result of differing prescription policies or practices between these hospitals

with the assumption that HJH and UH having restricted use of carbapenems compared to other hospitals. Another explanation could be different infection control protocols thereby leading to a reduction in risk for acquisition of hospital associated infections (HAIs).

KP resistance to piperacilin-tazobactam was high (165) across all sites. The high rates of ESBL-KP are worrisome as they limit treatment choices in the management of KP bactereamia. Resistance showed to be relatively lower at SBPAH and it is not entirely clear why KP resistance is generally lower at SBPAH compared to other hospitals, it can only be assumed that the possible reason would be due to differences in prescription policy at these different hospitals or differences in blood culture practices. (123)

6.4.10 Factors associated with MRSA and ESBL-KP

Several demographic factors were found to be significantly associated with antimicrobial resistance. For SA factors associated with lower odds of MRSA were: male gender, age-group <5 years and ≥60 years and hospital location (CHB, HJ, GSH and SBPAH). Factors significantly associated with lower odds ESBL-KP were older age ≥20 years and hospital location (SBPAH). Even though male gender had lower odds of ESBL-KP, the finding was not statistically significant. There is however no known reason that could explain such underlying associations. However, in European hospitals more males than females are treated at any moment in time. This is probably due to the more unhealthy lifestyles (more stress, more alcohol, more smoking) leading to earlier chronic disease in males than in females. In contrast, in the present study we had showed no significant variation in resistance pattern between males and females for ESBL in KP (male 67%, female 65%; p=0.282. Table 6.10) but MRSA was significantly more common in females (54%) compared with males (45%); table 6.9, p=0.0003), an indication that the situation may not be as observed in Europe.

The main limitation of this study is incompleteness of data, an inherent weakness of public health surveillance systems. Analysis of data stratified by the numbers of hospital beds of individual institutions was not done, constituting an important limitation of the study. In addition no data on admission date and occurrence of blood stream infection were available hence it was not possible to determine prevalence of nosocomial bactereamia. This being laboratory based surveillance therefore no data was available on clinical parameters and clinical outcomes and it was therefore not possible to ascertain the clinical impact of antimicrobial resistance on outcomes in these hospitals.

6.5 Conclusion

The rates of SA and KP resistance observed in this study are high. It is also of concern that there (i) is an increasing number of SA isolates resistant to vancomycin, (ii) are high rates of MRSA and (iii) is an emergence of carbapenemase-mediated resistance in KP which is rising above 3%.

It has been shown that appropriate antibiotic treatment reduces the likelihood of infections caused by antibiotic resistant pathogens and ultimately leads to improved patient outcomes. As such, to promote improved clinical management of bacteraemia episodes, antibiotic treatment guidelines and stewardship programmes should be developed based on local patterns of antibiotic resistance. Such a surveillance program is essential as it will contribute to ascertaining the precise burden of antimicrobial resistance among nosocomial infections at local (hospital) and provincial levels. Such data could provide a landscape for the enhancement of basic infection control practices and antimicrobial stewardship to combat the development and spread of antibiotic resistant bacteria.

Since these findings may not entirely represent the average resistance rate from all hospitals in South Africa because the study only covered hospitals from 3 out of 9 provinces, further studies are needed to confirm these findings as resistance rates seem to vary by geographical and hospital location.

Chapter 7   Comparative assessment of patterns of antimicrobial resistance of *Staphylococcus aureus* & *Klebsiella pneumoniae* blood culture isolates from GERMS–SA and CDW databases

This chapter presents comparative findings of patterns of antimicrobial resistance to various antimicrobial agents commonly used in hospitals. Comparative data presented here were from prospective (July 2010 to June 2011) and retrospective (July 2005 to December 2009) periods. The purpose was to ascertain validity and reliability of routine LIS data sources.

7.1 Introduction

A reliable antimicrobial surveillance system requires thorough assessment of data quality and control measures so as to ensure that blood culture data collected by the National Health Laboratory Service (NHLS) system through the Group for Enteric, Respiratory and Meningeal Disease Surveillance programme in South Africa (GERMS-SA) are reliable and representative of the South African population.

GERMS-SA conducts national laboratory-based surveillance of communicable diseases of public health relevance in South Africa. The surveillance system has been ongoing since the early 2000s'. All participating laboratories are asked to regularly send isolates of the selected pathogens following the well-defined case definitions. Such isolates are sent to GERMS-SA for further microbiological testing which includes culture and susceptibility testing, molecular testing, genotyping and phenotyping of the isolates etc.

To ensure appropriate data quality for the surveillance purposes, GERMS-SA endeavours to conduct data audits so as to evaluate the number of blood culture isolates received at the

reference laboratory compared with the number of blood culture isolates identified at the participating NHLS laboratories. The difference which can be expressed as a proportion can act as a proxy measure of how representative the GERMS surveillance network is in South Africa so that data aggregated and the results coming from such data can be generalized to the South African population with some degree of certainty.

A comparative assessment was conducted of blood culture data for *Staphylococcus aureus* (SA) and *Klebsiella pneumoniae* (KA) isolated in seven tertiary academic hospitals in South Africa. The aim was to assess completeness and hence reliability of the blood culture data that were entered into the GERMS-SA database from the sentinel laboratories. Furthermore, to simultaneously assess the reliability of routine blood culture data generated from the Laboratory Information System (LIS) by comparing such data with data collected in a more systematic and controlled manner by the ARSR unit of GERMS-SA enhanced surveillance system.

Antimicrobial susceptibility data from ARSR unit were uses as a gold standard comparator. It was assumed that GERMS-SA data are of more reliable quality since data are gathered through a rigorous research process following a specified standard protocol and subjected to error scrutiny. It was envisaged that such a comparative assessment would provide objective information on whether the data generated through the LIS despite its limitations would provide similar patterns of resistance, as that observed through analysis of blood culture data generated through the GERMS-SA surveillance system.

This assessment was not intended to evaluate functionality and performance of laboratories across different geographical location nor investigate laboratory standards. SA and KP are

bacteria of interest that were selected for the antimicrobial resistance surveillance by the GERMS-SA. This provided a platform for comparing validity and reliability of the CDW blood culture data that are routinely aggregated through the LIS.

7.2 Objectives

The specific objectives of the comparative assessment were:

- To ascertain that all blood culture isolates of selected pathogens under surveillance from the seven tertiary academic hospitals are reported to the GERMS-SA surveillance network.

- To assess the quality of antimicrobial susceptibility data of blood culture isolates of *S. aureus* and *K. pneumoniae* that are reported to the CDW to ascertain representativeness of the GERMS-SA surveillance data.

- To ascertain the proportion of missing data in order to determine the gravity of biased estimation of the prevalence of the blood stream infections caused by the *S. aureus* and *K. pneumoniae*.

7.3 Methodology

7.3.1 Study Setting

The study compared data obtained by ARSR of the National Institute of Communicable Diseases (NICD). Blood culture isolates of SA and KP were collected in 7 tertiary public hospitals associated with academic institutions. Details of participating sites have been reported elsewhere in this thesis. (Chapter 5, section 5.2.2)

7.3.2 Data extraction from the CDW

The procedure for data extraction from the Corporate Data Warehouse (CDW) of the NHLS followed the GERMS-SA protocol that is used when conducting data audits.

Blood culture data were accessed through a password controlled portal (https://cdwmicrostrategy.nhls.ac.za/MicroStrategy/asp/main.aspx), through use of legitimate log in details (username and password) followed by a selection of the bacterial pathogens of interest. For this study the pathogens selected were *S. aureus* and *K. pneumoniae* which are bacterial pathogens of focus for the Centre for Opportunistic, Tropical and Hospital Infections at the National Institute for Communicable Diseases (NICD). Details of how data were extracted from the CDW are provided elsewhere in this thesis (2.5 Data Management, Section 2.5.1).

7.3.3 Assessment of completeness of blood culture data from the CDW Database

The procedure for conducting the data audit from the CDW database followed the standard protocol developed by GERMS-SA quality assurance team. The process was done by specific matching parameters of blood culture data found in the CDW database with similar parameters in the GERMS-SA database, in order to identify non-matching cases for further scrutiny.

7.3.4 Matching of GERMS-SA Data to CDW data

To be able to match data in the two databases and identify missing isolates which were termed 'audits cases', we performed an automated matching using the VLOOKUP function

of MS Excel. This matching allowed for quick identification of similar records in the two databases, i.e. the GERMS and the CDW databases. Blood cultures that were done after 21 days from the date of the last blood culture were deemed to be a new bacteraemic episode, hence not regarded as duplicate records.

The records that did not match were put under further interrogation by using the manual eyeballing technique. This made it possible to further identify matching blood culture records in the two different databases. This was done by creating a 'Non-match' CDW spreadsheet to look the same as that of the GERMS-SA spreadsheet. This was achieved by aligning the order of the column in the two different databases, and then combining the spreadsheets and sorting by Surname, First Name, Collect Date and Reference Organism etc. The Non-match CDW cases that did not match the GERMS-SA cases, were identified as 'audit cases' and were coded "a" under AUDIT column in the CDW spreadsheet.

The list of all non-match cases which were called audit cases was then submitted to the unit laboratory manager for thorough checking of such cases on the DISAlab LIS. Once verified that such data were not duplicates, but were missing cases in the GERMS-SA database, data for such individual cases were entered onto the GERMS-SA antimicrobial resistance surveillance and research (ARSR) database. When all audit cases were identified and entered onto the database, a full quarterly surveillance statistical report was generated.

7.4 Results

7.4.1 Assessing data completeness

Table 7.1 presents an assessment report for a complete 12 months calendar period. The table highlights audit cases identified during the process of the surveillance audit of SA and KP

isolates from the 7 academic hospitals'clinical microbiology laboratories. By definition, audit cases were blood culture isolates that were found on the CDW database, but were not present on the GERMS-SA database, which means that these clinical isolates were not reported in GERMS-SA by the different laboratories. By implication, without this audit process, GERMS-SA surveillance is prone to under reporting the occurrence of SA and KP bacteraemia.

The distribution shows that the percentage of audit cases was highest at Charlotte Maxeke Johannesburg Academic hospital (29.3% and 36.4%, respectively) and lowest at Groote Schuur hospital (5.6% and 0.3%, respectively) for both SA and KP isolates. In general, there were fewer audit cases identified in the Western Cape Province clinical microbiology laboratories.

Table 7.1 Frequency distribution of audit cases identified for the period 1st January-31st December, 2011

		Audit Cases	
		Staphylococcus aureus N=409* (%)	*Klebsiella pneumoniae* N=365* (%)
Province	Hospital Name		
Gauteng	Charlotte Maxeke JAH	120 (29.3)	133 (36.4)
	CH Baragwanath Hospital	66 (16.1)	119 (32.6)
	Helen Joseph Hospital	45 (11.0)	35 (9.6)
	Steve Biko PAH	57 (13.9)	5 (1.4)
Free State	Universitas Hospital	55 (13.4)	46 (12.6)
Western Cape	Groote Schuur Hospital	23 (5.6)	1 (0.3)
	Tygerberg Hospital	43 (10.5)	26 (7.1)

*N= total number audit cases identified

7.4.2 Comparison of Antimicrobial Resistance data from GERM-SA and CDW data bases

We compared resistance patterns generated from the two data sources: 1) CDW which houses routine data and 2) GERMS-SA which houses data from reference laboratory where isolates from different NHLS laboratories are assessed and confirmed, and susceptibility testing undertaken in a more controlled research setting. Table 7.2 and 7.3 present the results of the comparative analyses of resistance patterns of the selected antibiotics for SA and KP. However it should be noted that differential sampling between the two study periods might have introduced selection bias in comparing resistance proportions estimating antimicrobial resistance.

The cloxacillin mean resistance rate was 15.4% (range 0.4% - 43%) (Table 7.2). Gauteng province hospitals had very low resistance (range 0.4 - 5%), so when excluding data for cloxacillin from Gauteng Province hospitals, cloxacillin mean resistance rate was 39% (range 30-43%). The mean resistance rate as well as the range of antimicrobial resistance has minimum variation from each other between the GERMS-SA and the CDW data sources. The table gives a picture of the pattern of antibiotic resistance among commonly used agents for treating *S.aureus* bactereamia.

Table 7.2 Comparison of rates *S. aureus* resistance for periods 2005-2009 and 2010-2011

Antibiotic	CDW 2005-2009	GERMS-SA 2010-2011
Ampicillin	95.7 (88.2%-96.5%)	94.7 (89.4%-98.0%)
Cloxacillin*	15.4 [(30%-43% (UH & WC), ^GA 0.4-5.0%)]	54.1 (36.3%-83.2%)
Clindamycin	32.4 (15%-72%)	41.3 (28.4%-57.2%)
Erythromycin	34.5 (19%-44%)	49.7 (31.6%-75.7%)
Gentamicin	51.8 (22.2%-66.7%)	55.1(33.5%-85.5%)

*UH and Western Cape Province hospitals (resistance range 30.4-43.0%); ^Gauteng Province hospitals (resistance range 0.4%-5%)

In Table 7.3, the rates of antibiotic resistance between the GERMS-SA and the CDW data sources also shows very minimal variation amongst the tested antibiotics. Comparatively, more cases of carbapanemes resistance were identified during the 2010-2011 period: ertapenem 50 resistant isolates out of 2474 KP isolates tested; imipenem 4 out of 3059 isolates tested; and meropenem 5 out of 3046 tested during the 2005-2009 period. While in the 2010-2011 period 30, 36, and 14 resistant isolates to etrapenem, imipenem, meropenem, respectively, were identified out of 1045 KP isolates tested.

Data in the table7.3 suggests an increase in carbapanem resistance during the period 2010-2011 which might have been as a result of an enhanced ability to accurately determine resistance in all isolates as compared to previous years. However, when one looks at the data on carbapenem resistance in table 7.3, it can be seen that the ranges presented for the GERMS-SA carbapenem resistance are much wider that those recorded in the CDW, suggesting the possibility of a technical or recording problem with the GERMS-SA (2010-2011) over the one-year period. Despite this observed increasing resistance, the findings between the two databases show a minimal variation in terms of KP resistance pattern among these antibiotics.

Table 7.3 Comparison of rates of *K. pneumoniae* antimicrobial resistance for periods 2005-2009 and 2010-2011

Antibiotic	CDW 2005-2009	GERMS-SA 2010-2011
Amoxclav	64.8 (52.7-73%)	64.4 (50.0-72.6%)
Ciprofloxacin	51.8 (43-77%)	44.7 (31.8-59.7%)
Ertapenem	2.0 (0.8-4.6%)	2.9 (0.0–6.3%)
Gentamicin	58.7 (53-70%)	62.8 (54.3-75.0%)
Imipenem	0.1 (0.0%–0.3%)	3.4 (2.0–8.1%)
Meropenem	0.2 (0-0.2%)	1.3 (0.0–3.2%)

7.5 Discussion and Conclusion

This comparative assessment of blood culture data showing rates of antimicrobial resistance was carried out in order to validate the effectiveness of the CDW data repository as a useful and effective tool that can be utilised to monitor antimicrobial resistance in South Africa. In conducting the audit, we were able to use the CDW as a proxy with which to assess the validity and reliability of the LIS as an effective system that gathers reliable blood culture data that can be systematically analysed to provide valid evidence of the existing patterns of antimicrobial resistance to nosocomial pathogens in tertiary public hospitals. For antibiotic resistance to be effectively controlled, an effective surveillance system that uses existing routine systems of blood culture data collection needs to be established. This system, if put to use would provide regular updates on trends and patterns on antibiotic resistance amongst nosocomial blood borne infections.

In South Africa, the well established LIS that forms a network of NHLS laboratories serves as a solid platform on which to institute regular analysis of blood culture data gathered routinely from the network of public sector clinical microbiology laboratories. We, therefore, needed to assess the validity and reliability of blood culture data produced through the routine system and understand if such data provides similar results to that gathered in a systematic way through a rigorous research process.

From the results provided above, it is clear that laboratories in the Western Cape Province are better able to report isolates of SA and KP as shown by few missing isolates, i.e. isolates identified by the routine laboratory procedures but not reported to GERMS-SA surveillance system. In addition to that, the results provides a comparative basis on the reliability of

routine data acquired through the CDW as mean rates of resistance for various antibiotics showed minimal variation. It was however questionable why MRSA resistance in Gauteng hospitals (0.4-5%) showed to be lower (as reported in Chapter 5, Table 5.3) than the mean estimated rate of resistance shown by the systematic review (33%),(31) and the prospective analysis (54.1%).(Chapter 6, Figure 6.1) The phenomenon of antibiotic suppression carried out by the clinical microbiology laboratories in Gauteng Province might be the plausible explanation for such a variation in the reporting of MRSA. The practice of "antibiotic suppression" is designed to guide clinicians on the choice of appropriate antimicrobials in accordance with hospital policies; therefore susceptibility status of bacterial isolates is much more likely to be reported on. However susceptibility test information relating to isolates that are resistant to other antimicrobials which are not only the preferred agent within an antibiotic class according to the prevailing policies are likely to be suppressed.

From these findings, it can be concluded that clinical microbiology laboratories in the Western Cape and Free State Provinces might have employed more effective recording practices leading to fewer recording errors in their reporting of susceptibility results hence better reporting mechanism for nosocomial bacteria i.e. SA and KP isolates as opposed to laboratories in the Gauteng province. We can also conclude that the observed MRSA rates for Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath Hospital, Helen Joseph Hospital and Steve Biko Pretoria Academic Hospitals (Chapter 5, Table 5.3), might be an indication of systematic error in the reporting of data by the laboratories, therefore might have been systematically underreported hence might not be reliable representation of MRSA rates in these hospitals. An in-depth inquiry might be essential to determine the validity of such data.

Chapter 8   Understanding Laboratory Methods and their impact on antimicrobial resistance surveillance in Muhimbili National Hospital, Dar es Salaam, Tanzania

This chapter describes the findings of a laboratory audit of the procedures and practices of the microbiology laboratory at Muhimbili National Hospital. Critical assessment of the sequence of events pertaining to blood cultures from point of collection in the wards to the time results are released to the clinicians for viewing has been outlined. In addition blood culture data entry into the JEEVA LIS and challenges of manual data entry has been described.

8.1 Introduction

Muhimbili National Hospital's (MNH) existence dates back to 1910 when it was known as Sewahaji. It is a 900 bed specialised National Referral and University Teaching Hospital (175) that provides tertiary health services to inhabitants of Dar es Salaam region, Tanzania, with an estimated population of 2.5 million people. (176) The hospital admits 1,000 to 1,200 in-patients per day. Blood cultures at this hospital are not routinely conducted because clinical diagnosis of bacteraemia and empirical antibiotic therapy is the main approach to clinical management. Blood culture is only requested in special circumstances, mostly due to treatment of non-response or in neonatals due to non-specifity of clinical symptoms in this age group. The aim of this study was to describe in details laboratory methods and procedures relating to blood cultures and their potential impact on antimicrobial resistance surveillance among nosocomial bacteria.

8.1.1 The Central Pathology Laboratory

The Medical Laboratory Services in Tanzania (called Tanganyika during the colonial era) were established in the late 19th Century during the German administration. The first Government Health Laboratory was established in 1897, at Ocean Road in Dar es Salaam. Historically, this laboratory was the first site of a medical laboratory in Tanzania. The laboratory was often visited by Dr Robert Koch who worked in the laboratory on several occasions as he was investigating tropical diseases such as malaria, sleeping sickness etc, which were then a major health problem in the country. Laboratory services have grown and expanded country wide. The Ocean Road Laboratory became the Central Pathology Laboratory (CPL) in the early 1960s and is still operational under the Ministry of Health and Social Welfare. (177) The CPL, located at the MNH, is a key player in the provision of high quality laboratory services to all patients referred to and admitted at MNH or attended to as out-patients.

8.1.2 Departments and Laboratory information system

The CPL is the leading provider of diagnostic laboratory services in Tanzania. In addition, the CPL offers referral laboratory services for tests requests from other public and private hospitals within Dar es Salaam and surrounding regions. Among the services provided by the CPL are: microbiology, histopathology, parasitology, haematology and blood transfusion, clinical chemistry etc. The CPL uses a laboratory information system (LIS) fully interfaced with all automated diagnostic machines and hospital information management system (HIMS), the Jeeva system 2000. This was established as an attempt to improve turnaround time for laboratory results.

All clinical departments are computerised and interlinked to the LIS and the results are entered and posted on the Jeeva LIS for clinicians to access directly in the wards and other clinical departments through logging into the system with their username and password. The clinicians view the results online in the wards, and this expedites the clinical decision regarding treatment modalities for bacteraemia cases. Hard copies of the laboratory results are sent to the wards afterwards for purposes of filing in the patient's files and cross referencing in case of a future episode of an illness. The microbiology unit at the CPL handles high volumes of laboratory results ranging from samples of blood, cerebral spinal fluids, pus swabs, urine specimens, stool etc. The microbiology unit does the following tests among others: bacterial identification, antibiotics susceptibility testing and serological tests. The LIS helps to ensure that results are captured in time and transmitted or released to the patients within acceptable time limits. (178)

8.2 Methodology

8.2.1 Design and study setting

A systematic audit of blood culture procedures and practices was carried out in the department of microbiology of the Central Pathology Laboratory of Muhimbili National Hospital. The audit lasted 3 days and focussed on the procedures and practices carried out in the process of dealing with blood cultures i.e. tracing a pathway from receipt of blood culture specimen in the microbiology laboratory to processing the blood culture to communicating results to the clinicians in the wards and entering results on LIS. Our study focussed at blood culture from bacteraemia caused by *Staphylococcus aureus* (SA) and *Klebsiella pneumonia* (KP).

8.2.2 Data collection procedures

We used a standard guide (Appendix 12.4) as we went through different sections of the microbiology department focussing on how blood cultures are performed and how data are gathered in the laboratory and utilised for surveillance. The audit involved i) a comprehensive orientation on the activities of the bacteriology section, to familiarise with standard routines and laboratory practice, ii) observation of how blood procedures are done in the laboratory and, iii) individual discussions with staff involved in technical procedures of blood culturing and data entry of blood culture results.

8.3 Results

8.3.1 Blood culture specimen flow

We schematically describe in the chart below specimen flow of blood cultures and related procedures pertaining to blood cultures at MNH.

Figure 8.1 Blood culture data flow and interlinkage with the LIS at MNH microbiology laboratory

Step 1: Two blood culture bottles are collected and sent to the laboratory. For children, only a single specimen is collected into a special blood culture bottle. Duplicate specimens in this laboratory are rare as blood cultures are collected on special request only not as a routine test.

Step 2: From the wards, all specimens are delivered to the laboratory reception area where they are sorted out based on the type of the specimen.

Step 3: At the reception area, blood culture specimens are isolated from the pool of other specimens by the laboratory clerk responsible for all microbiology specimens. A serial number is allocated and pasted onto each of the blood culture specimen bottle.

Step 4: The laboratory clerk then enters patient demographic details from a specimen order form into a register book and LIS database. Once this is done, the specimen is delivered to the bacteriology laboratory for processing.

Step 5: In the bacteriology laboratory, the technician receiving the specimen then enters the patient's details into yet another register book so as to track samples and minimise loss.

Step 6: The blood culture specimen is then placed into the HERA CELL 150 incubator and physically monitored each morning to detect bacterial growth. If visible signs of positive culture are noted, the specimen is taken out for Gram staining and susceptibility testing.

Step 7: The results of blood cultures (both positive and negative specimens), are documented on the blood culture results form, which is then attached to the original laboratory request form.

Step 8: Verification of blood culture results is done by the Microbiologist who heads the department or his immediate representative. Once results are signed off, the results are ready to be released to the wards.

Step 9: The blood culture results are handed back to the laboratory clerk, who manually enters them into the JEEVA LIS. The electronic record is linked to the ward in such a way that the clinicians in the ward can access the results directly online, through the LIS computer network installed in the wards. The hard copies of the results are also sent to the requesting clinician. (See an example of MNH laboratory request form, Appendix 12.6)

Step 10: The blood culture specimen is discarded after 5 days once no indication of positivity is observed. All necessary protocols for blood culture are followed so as to minimise errors.

8.3.2 Sample volumes

Muhimbili National hospital is a large and busy hospital that serves approximately 1,500 out patients per day. However, the number of blood culture specimens received each day by the microbiology department, is in the region of 25 – 30 thus providing a clear indication that blood culturing is not a routine practice. In the wards, blood culture is only requested in specific clinical circumstances such as failed empirical antibiotic treatment. As per information from the laboratory register, it was clear that more blood culture requests originate from the paediatrics department's neonatal unit.

8.3.3 Blood culture processing

Susceptibility testing of isolates is dependent on the availability of disk panels and therefore, not all isolates are tested for resistance to all antibiotics. Sometimes, testing is only done on second line drugs which are not the standard of care in the hospital. The results of these tests are therefore, of little help to clinicians who manage patients with blood borne infections. It

was observed that all *Staphylococcus aureus* isolates were tested for vancomycin resistance, as a way of monitoring emerging vancomycin resistant *Staphylococcus aureus* (VRSA).

8.3.4 Common antibiotics tested

The most common antibiotics subjected to susceptibility testing of blood culture isolates at this hospital were:

Staphylococcus **species**: amikacin, penicillin, ampicillin, cloxacillin, tetracycline, erythromycin, gentamicin, cephalothin, chloramphenicol, vancomycin.

Klebsiella **species:** ampicillin, chloramphenicol, tetracycline, amikacin, amoxyclav, cefuroxime and imipenem.

Pseudomonas **species**: ampicillin, amikacin, ciprofloxacin, chloramphenicol, gentamicin and cotrimoxazole.

Escherichia coli: chloramphenicol, gentamicin, amikacin, ampicillin and cefuroxime.

8.3.5 Antibiotic susceptibility testing

The antibiotic susceptibility testing procedures at this site are done in accordance with the Clinical Laboratory Standards Institute (CLSI), 2010 guidelines. The minimum inhibitory concentration (MIC) for each antibiotic to determine cut off for antibiotic resistance as outlined in these guidelines are followed. External quality control is done on a regular basis using specimens from the Centres for Disease Control (CDC) to ascertain validity and reliability of antibiotic susceptibility results produced by this laboratory.

8.3.6 Challenges in blood culturing

8.3.6.1 Automated laboratory equipment

Lack of a functioning automated microbial detection system was observed to be important obstacles to effective blood culturing at this laboratory. The automated blood culturing equipment is often not in good working order and servicing takes a long time to be done. The laboratory often relies on manual blood culturing technique which has its own limitations, such as subjective determination of a positive culture through visual assessment. Accuracy is dependent on individual technician's visual acuity, hence subject to over or under estimations of true positives. However, manual blood culturing is still the most common mode of blood culturing in most resource constrained countries.

8.3.6.2 Blood cultures results

As per records entered into the blood culture register book, high rates of negative cultures were observed. This might be due to prior antibiotics used before a blood culture specimen was taken or it might be a true representation of negative blood cultures. In addition, we also noted that there was a high rate of coagulase negative *S.aureus* which might be due to contamination of the blood culture samples at the point of collection.

8.3.7 Common challenges and errors in blood culture data recording

The outline here gives some of the common challenges and errors in data recording found at MHN microbiology laboratory.

- There was lack of a standardised way of entering data. For example, age can be entered as date of birth, age in months, days, years, etc. It was also often just documented as adult or

child. This created confusion in terms of knowing the exact ages of the patients who had blood cultures done.

- Missing data was a major issue, as information on gender, age, hospital ward, type of organism and clinical data was often not available.

- Lack of standardised reporting of the blood culture results i.e. results would be reported in the following ways: no bacterial growth; +ve. Neg, -ve, NEGATIVE, NBG etc.

- Mixture of data type entered and only a few had sensitivity results entered.

- Lack of specific dates that specimens were taken e.g. the record would just show July: but no date was specifically mentioned. (i.e. which date in July?)

8.4.8 Standard operating procedures (SOPs)

The microbiology department operates on principles laid down in the standard operating procedures (SOPs) manual. These procedures are overseen by a Quality Control Office, who is a member of the team in the microbiology laboratory. The controlling officer is responsible for effecting and approving any changes to the SOPs. Implementation of the SOPs is overseen by the microbiologist, who is the head of department. The manuals are kept in the microbiology laboratory for ease of reference by all team members.

8.4.9 Challenges with data quality

8.4.9.1 LIS data entry format

Entry of blood culture results into the JEEVA LIS database was done by a single individual. There was no verification of data entered by a second individual to check for accuracy of data entered and to allow for timely correction of errors. The system does not have check codes to

control data that is being entered. For example, a characteristic such as 'age' the system could take in data in any numerical format such as absolute age, year/date/month, year, months and days. This was certainly problematic and a huge source of error.

8.4.9.2 Clinical data

There was often no documentation of patient's prior antibiotic use, before a blood culture sample was taken. No provisional diagnosis was captured on the laboratory request form. Should the laboratory request form have some clinical history documented, such information would not be captured onto the system, as the database structure of the LIS was not programmed to capture such information.

8.4.9.3 Determination of nosocomial bactereamia

There was no documentation on the laboratory request forms on duration of in-hospital stay prior to blood culture specimen being taken. Lack of this information makes it difficult to separate nosocomial from community acquired bacteraemia. In so doing, the burden of antimicrobial resistance due to nosocomial infection becomes difficult to effectively ascertain.

8.5 Discussion and Conclusion

Surveillance of antimicrobial resistance is primarily dependant on good laboratory procedures, good quality and reliable routine blood culture data. To improve the quality of blood culture data and minimise improper estimates of antimicrobial resistance, it is essential that important steps be taken to improve the system of specimen collection at the point of care, registration and blood culture procedures in the microbiology laboratory. High rates of specimen contamination, as evidenced by more coagulase negative culture results shown in

section 8.3.6.2, 'blood culture results' calls for the need to, proactively, improve blood culture specimen collection procedures as this would ultimately lead to a reduction in blood culture contamination, and provide proper estimates of bacteraemia episodes and rates of antimicrobial resistance.

We also need to place special emphasis on appropriate completion of blood culture request forms by clinicians in the wards, specimen registration by laboratory clerk and accurate entry of blood culture results by laboratory technician. Accuracy of blood culture results could also be improved if the automated blood culture machines were functioning properly. Improvements in quality of data could also be enhanced through improved data entry process into LIS either by introducing another software such as WHONET free access software developed since 1989 by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance specifically for antimicrobial susceptibility monitoring plus introducing a system of validating data entered into the LIS. (80)

The LIS need check codes to be built in, so that the system also helps to track errors on data entry. Simple improvements in the current system could update the system to be an effective surveillance tool to help monitor development and spread of antimicrobial resistance among blood borne pathogens in Tanzania. Such information in the long run will help in policy formulation around antimicrobial usage to contain the growing crisis of antimicrobial resistance in the country.

Chapter 9 Discussion

This chapter provides a detailed discussion of the relevant findings of the study. Firstly, a comprehensive summary of the study findings is presented followed by a detailed discussion of each of the findings and comparing these to what is found in the global literature. This is followed by a description of potential measurement errors focusing on bias and confounding which might have led to over or underestimation of the resistance pattern observed in this study. In this chapter strengths and major limitations of the study methods are discussed as well as strength and limitations of the existing LIS as an appropriate tool for monitoring antimicrobial resistance. In closing, a summary of key issues discussed and suggestions for improvement of the existing system are provided.

9.1 Introduction

Surveillance of antimicrobial resistance aims to improve the detection, monitoring and characterisation of resistant strains in humans with SA, KP and PA bacteraemia among others.(179) Thus the study focussed on these pathogens as they were common hospital acquired bacterial infections and were prone to antibiotic resistance. (103, 134, 180) Identification of resistant isolates through use of routine laboratory data will allow effective interpretation and mapping of trends and patterns of AMR, thus leading to formulation of strategies to prevent and/ or control development and spread of such resistant isolates.

'Combat Drug Resistance' was the theme of the World Health Day on 7 April, 2011 aimed at raising awareness and putting across a six-point policy package to combat the spread of antimicrobial resistance. The focus was "No action today, No cure tomorrow", so that

globally we should all move and invest our resources towards minimising the development and spread of antimicrobial resistance. (181) The WHO six-point policy included: i) commit to a comprehensive, financed national plan with accountability and civil Society engagement; ii) strengthen surveillance and laboratory capacity; iii) ensure uninterrupted access to essential medicines of assured quality; iv) regulate and promote rational use of medicines, including in animal husbandry, and ensure proper patient care; then reduce use of antimicrobials in food-producing animals; v) enhance infection prevention and control; vi) foster innovations and research and development for new tools. (181)

Antimicrobial resistance is an old problem. However, due to increasing spread, it has become a daunting public health problem requiring urgent and consolidated efforts in order to avoid the world from regressing to the pre-antibiotic era. (181) Antimicrobial resistance occurs when microorganisms such as bacteria, change in ways that render the medication used to cure the infection they cause ineffective. This poses a major concern since resistant infection may cause death, can spread across the community forcing individual and society at large to incur heavy costs in caring for relatives suffering from a disease of an infectious origin caused by a resistant bug.

Antimicrobial resistance is facilitated by many factors including: inappropriate use of medicines, e.g. in circumstances where a prescribed dosage is inadequate or a patient is non-adhering to the prescribed dosage. In addition, low quality antibiotics, incorrect prescriptions and poor hospital infection control, are all factors that promulgate the development and spread of antimicrobial resistance. Lack of commitment from ministries of health to address the issues highlighted above, poor surveillance systems as well as lack of tools to diagnose,

treat and prevent spread of these superbugs, all obstructs effective control of antimicrobial resistance. (181)

This thesis was done to contribute towards this fight against the spread of resistant bugs. The study focussed on assessing the relevance and utilisation of laboratory based surveillance in curbing the development and spread of antimicrobial resistance as per the WHO call alluded to earlier. (182) To establish whether the LIS can be effectively used for antimicrobial resistance surveillance, we examined in great detail routine blood culture data investigating rates of resistance, trends and patterns of antimicrobial resistance including distribution and risk factors associated with resistance specifically focusing on SA, KP and PA bacteraemia among patients in South Africa.

Our study looked at single episodes of bacteraemia amongst patients attending and admitted at public tertiary hospitals associated with academic institutions in the provinces of Gauteng, Free State and Western Cape. Of interest were rates of MRSA and ESBL-KP, as well as PA resistance to polymyxins. These organisms were of prime interest due to their association with hospitalization and high rates of antimicrobial resistance. (183, 184) PA is known to commonly be multi-drug resistant, but this was assessed based on retrospective data only.

For us to contain antimicrobial resistance, some of these strategies should be introduced: surveillance, infection prevention and control, product development as well as regular research. (185) In this thesis, we mostly focused on surveillance as an effective method to reduce the development and spread of resistant isolates in the population. Knowledge of antimicrobial resistance guides or determines the choice of antimicrobial therapy during an episode of bacteraemia.

9.2 Evaluating a Public Health Surveillance System

The CDC defined surveillance as: "the ongoing systematic collection, analysis, and interpretation of data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those responsible for prevention and control." In this thesis we have used antimicrobial resistance as example of such surveillance.

This section highlights the pros and cons of the currently used JEEVA operated LIS as a possible effective tool for antimicrobial resistance surveillance. As reiterated by Langmuir A.D.,*"Good surveillance does not necessarily ensure the making of right decisions, but it reduces the chance of wrong ones."*(186) The parameters for evaluating a public health surveillance system are described below focussing on surveillance of antimicrobial resistance and making reference to specific laboratories that were part of the study.

Simplicity: Data collection is on-going as part of the service provision. This will only require improvement in using available resources.

Flexibility: The system will need modification of data entry parameters within the existing data collection structures.

Data quality: Manual entry of data is subject to errors and omissions. Also, missing data is a major challenge.

Acceptability: Upon obtaining permission from relevant authorities, data can be made available to other people.

Sensitivity: Susceptibility based on culture procedures follows the international acceptable standards of antimicrobial susceptibility testing.

Positive Predictive Value: Resistance and or burden of blood borne pathogens can be assessed monthly/yearly using available data.

Representativeness: Blood culture samples were from patients within certain defined geographical areas, i.e. Gauteng, Western Cape, Free State provinces in South Africa.

Timeliness: Turn-around time is difficult to determine as time in and time out is not recorded in the LIS. However, time when results are made available is documented hence it can be determine how many days it took for the blood culture results to be released.

Stability: The surveillance system in our case is well established, as it is ongoing, and involving routine data collection of service related data.

9.3 Surveillance of Antimicrobial Resistance

Continuous monitoring of antimicrobial resistance would allow quantifying the magnitude of antimicrobial resistance and demonstrate what public health challenge it poses. Such monitoring would also help to track down emerging resistant bacterial strains. Surveillance would also help to reinforce identification and molecular characterisation of bacterial strains. Such an undertaking would ultimately help to contain new resistant strains but also allows for systematic comparison of data across sites. (137) Data from a surveillance model also helps to assess effectiveness of interventions implemented in hospitals, such as the hospital infection control programs, to determine if the intervention was associated with reduction in prevalence of resistant bugs such as MRSA.

Our analysis demonstrates that routine antimicrobial susceptibility tests that are performed on a daily basis in the clinical microbiology laboratories, despite their subtle shortfalls, are a major source of data for antimicrobial resistance surveillance and produce comparable results of resistance patterns with data collected in a research environment. However, it should be

taken into account that the quality and reliability of the routine data are usually uncertain. To achieve and derive satisfactory results of estimates of resistance patterns, efforts need to be invested in terms of quality control procedures to improve the overall quality of blood culture data. In this study only the first isolate per patient was included in the analysis. In addition, in order to facilitate comparison of rates of resistance between different hospitals/ geographical areas, the LIS needs to set on a common file format for analysis, (132) such as the DISALab platform across NHLS sites.

9.4 Burden of MRSA & ESBL

Nearly all strains of SA in South Africa are resistant to penicillin, and >30% up to as high as 80% (range 30.4-98.8%) are resistant to methicillin-related drugs (Chapter 5, Table 5.3 and Chapter 6, Table 6.7). MRSA rates in this range are similar to those reported from North America and Europe. (187) Significantly lower rates of MRSA were observed at SBPAH, which might possibly be due to sampling bias of MRSA isolates obtained from different hospitals. Vancomycin for many years has been the main stay and effective treatment for the clinical management of methicillin resistant strains. However, there have been reported strains of SA isolates resistant to vancomycin (VRSA) (Chapter 5, Table 5.3 and Chapter 6, Table 6.2).

In 2006, only 1 isolate was reported (Chapter 5, Table 5.4) while in the period July 2010 and June 2011, 9 isolates of vancomycin intermediate *S.aureus* (VISA) were identified, (Chapter 6, Table 6.2) an indication of emerging development of glycopeptide resistance among SA isolates in South African tertiary public hospitals. The magnitude of methicillin-resistant SA is estimated at ~560 invasive isolates per year from the 7 tertiary public hospitals in three provinces in South Africa, July 2010-June 2011 data, (Chapter 6, Table 6.3). MRSA rates in

this range are high, compared to present resistance rates of <20% for European countries, including those from southern Europe. (188, 189) However such a similar trend was also observed in the USA as reported by Klevens et al. (187)

The mean rate of ESBL-KP was 74.25% while the rates for UH and SBAH were 81.3% and 62.4% respectively. Although numerically the rates were statistically significantly different (p= 0.022), from a treatment and infection control point of view the figures are all high hence cause for alarm. Such geographical differences have been reported from previous studies which also documented ESBL-KP rates as low as 13.5% (95% CI 12.8%-14.1%) among KP isolates. (190, 191) Separate analyses of factors associated with antimicrobial resistance were performed for both retrospective and prospective data to determine which demographic characteristics were significantly associated with resistance.

Analysis of retrospective data to carry out risk factor models focused on resistance to one or more of the standard antibiotics used in clinical practice and for prospective data, we streamlined our analysis to focus at factors associated with MRSA and ESBL-KP isolates. In the prospective analysis, adjusting for other factors in the multivariate model, age <5 years, male gender, and hospital location showed to be significantly associated with MRSA. ESBL-KP resistance was associated with factors such as age <5 years and hospital location.

9.5 Representativeness of the study sample

The populations included in the univariate and multivariate analysis were cases of clinical isolates from patients who were admitted in hospitals that fell within our study sites. All cases that had a positive culture for SA, KP, and PA with available data on susceptibility testing were included. We compared susceptibility test results of such cases between different

demographic factors and hospitals. This was done to assess whether distribution of the case population differed by these characteristics. All isolates included in this study originated from enhanced surveillance areas which form part of GERMS-SA surveillance network.

There were some differences in the proportion of isolates from these sites, with higher proportions of SA isolates from Helen Joseph, Tygerberg, and Groote Schuur hospitals than KP and PA. The proportion of KP isolates, were greater at CHBH and SBPAH than SA and PA, while the proportion of PA isolates were greater at SBPAH and CHBH than SA and KP. The proportion of individual isolates was higher than the mean distribution of isolates for the 7 hospitals included in the study. This could suggest a possible excess of SA, KP, and PA cases, but also may be an indication of differences in effectiveness on hospital infection prevention programs between hospitals.

The rates of antimicrobial resistance were different between three provinces with a higher rate of SA, KP, PA observed in Gauteng followed by the Western Cape and lowest in the Free State. This might be due to the fact that these provinces are mostly in the urban settings with bigger tertiary hospitals and dense populations. The other reason could be differences in clinical practice regarding blood culture procedures or differences in hospital protocols regarding blood cultures specimen collection practices with other settings being unrestricted in terms of doing blood cultures or having over diagnosis of clinical bacteraemia by some clinicians who are more likely to order or do blood cultures.

The proportion of antibiotic resistant isolates was different between age-groups with greater proportions of antibiotic resistance noted in the <5 years age group. The reason might be due to high utilization of antibiotics in this age group since more bacteraemia episodes occur in

the under-five children compared to older children and adults. This is due to high colonization of SA, KP and perinatal acquisition of PA during birth, and immature immune function making children, particularly neonates, at higher risk of bacteraemia. (192)

The proportion of males with resistant isolates was generally greater than females, which could be due to the fact that there were more isolates of SA, KP and PA in males. On the other hand, this could be a sign that occurrence of episodes of bacteraemia due to these pathogens is higher in the male population. Such differences might also be a sign of preponderance for bacteraemia due to these organisms among males, but could presumably also be due to selective blood culturing that favours the males population.

In summary, there were relative differences in distribution of resistant isolates due to SA, KP and PA by geographical location (province, hospital) and demographic factors (age, gender) across the duration of the study. Such differences may limit generalizability of findings and of patterns of resistance to other hospitals or provinces. Our main interest was analysis of trends and patterns of antimicrobial resistance of pathogens commonly associated with hospitalization. The purpose was to assess, if the LIS at NHLS could be a sufficient tool to be effectively used to monitor resistance. To achieve that, we focused on data from the 7 hospitals collected retrospectively and prospectively investigating the quality and availability of data on antimicrobial susceptibility test results.

To ascertain reliability of the LIS as an effective tool to determine patterns and trends of resistance, completeness of routine susceptibility data were important to the study. Even though data were incomplete for most of the antimicrobials that had undergone susceptibility testing, data for retrospective analysis did produce results that were compatible with previous

findings. (31) Examining the proportion of isolates across the three organisms, there were no obvious incorrect findings to suggest major errors in data entry or selective blood culturing. However there were discrepancies between the hospitals from Gauteng, Western Cape and Free State in terms of MRSA proportions.

The proportions are pretty similar for all the three organisms despite subtle differences seen. Even though our study sample was convenient, it appears homogeneous and relatively representative of all patients admitted with bacteraemia due to SA, KP, and PA to these hospitals. Therefore, we can say with a certain degree of confidence that it is not very likely that the validity of our findings of trends, patterns and distribution of antimicrobial resistant isolates presented in this report could have been underestimated.

9.6 Systematic overview of study findings

9.6.1 High rates of resistance to antimicrobial agents

9.6.1.1 Staphylococcus aureus

Antimicrobial resistance is a public health problem that is well recognised globally. (193) Infection caused by *S. aureus,* particularly MRSA, has been increasing worldwide since its first discovery in a British hospital. (194, 195) Our data subscribes to these observations from previous studies, and reports a crude MRSA rate of 15% in the 2005-2009 period using retrospective routine laboratory data (due to antimicrobial suppression policy). This was however inconsistent with a crude rate of 54% observed with prospective data collected through an active surveillance mandate in the same hospitals over a 12 months period (July 2010-July 2011).

MRSA propagates therapeutic challenges in the management of patients; is a major cause of morbidity and mortality; and leads to high costs of health care services due to long hospitalisation and use of vancomycin, which is a more expensive alternative treatment. (196, 197) There is however a growing worry due to emerging vancomycin resistant SA strains similar to observations of Appelbaum. (198) Our data showed emerging vancomycin-resistant SA in South African hospitals. While only 1 isolate was reported in the 2005-2009 retrospective data, 9 isolates were identified in the prospective analysis over a 12 months period (8 of them reported from the hospitals in Gauteng province). MRSA varied and significantly differed between hospitals (p = <0.001).

This confirms observation from previous studies that showed that prevalence of MRSA differs widely between countries and among different hospitals in the same country. (147, 199, 200) Increasing prevalence of MRSA might be due to differences is prescribing practice or antibiotic controls between hospitals or differences in infection control practices or may be inherent geographical differences in genetic characteristics such as the *mecA* gene, which encodes an altered penicillin-binding protein (PBP 2a), a membrane-bound protein. This is a key genetic component responsible for resistance which is not native to the *S.aureus* genome. (201, 202)

In our study, despite challenges in the quality of retrospective data that we extracted from the CDW, the analysis showed a significant downward trend of MRSA from 22.2% in 2005 to 10.5% in 2009 (p=0.042). Because of the systematic error in recording MRSA in Gauteng hospitals by "antibiotic suppression" of data, the apparent decline of MRSA over the study period (p=0.042) cannot be accepted at face value. In the case of a real decline in MRSA this could have been as a result of an improvement in infection control practices in tertiary

hospitals over this period. This could potentially be a real decline in MRSA in South Africa, a sign that there might have been an improvement in antimicrobial prescription control or in hand washing practices in these tertiary hospitals over this period. A similar pattern was reported by Adam et al. in a study done in Canadian hospitals where they showed a drop of MRSA rates from 26.7% in 2007 to 19.8% in 2009. (115) This shows that there is sufficient evidence from developed countries that improved infection control led to a decline in MRSA in hospitals. This is an indication that should a similar intervention have taken place in SA, it might have led to a similar observed outcome.

9.6.1.2 Klebsiella pneumoniae

There is an increase in carbapenem-resistant *K. pneumoniae* isolates worldwide. (134) Our study reported imipenem resistance among KP of 0.4% in 2005 to 4.0% in 2011. The pattern is consistent with what was reported by Braykov et al. (190) in the USA showing an increasing trend of carbapenem-resistant *K. pneumoniae* from 0.1% to 5.4% between 2002 and 2010; and from 1-2% in the years 2006-2009 to 15% in 2010 in Italy. (203) The frequency of resistance to extended spectrum of cephalosporins in our study was above 70%, which is much higher than what was reported by the Braykov et al, (190) study, reporting rates of 5.3% - 11.5% between 1999 and 2010.

This is an indication of a more serious challenge of increasing resistance to extended spectrum of cephalosporins in South Africa compared to the USA. These pathogens were traditionally endemic in hospitals but are posing a challenge as they might slowly be spreading to non-health care settings. The spread of these organisms is usually facilitated by patient mobility as they get transferred from one facility to the other such as from long term

care facilities, which happen to be a breeding ground for antimicrobial resistant bugs. (204, 205)

In addition to challenges with spread of antimicrobial resistance, carbapenems are reserved as treatment of choice for severe infections caused by ESBL-producing organisms making the global emergence of carbapenem-resistant *Enterobacteriaece*a strains a cause of great public health concern. (206, 207) Carbapenem-resistant *K. pneumoniae* pockets of outbreaks have also been described worldwide (208, 209) and have been reported as a common type of carbapenem-resistant *Enterobacteriaece*a in North America. (210, 211)

Due to increasing rates of resistance and associated therapeutic challenges of clinically managing carbapenem-resistant *Enterobacteriaece*a, there is therefore an overemphasis to intensify monitoring spread of resistant pathogens. This underscores the value of routine antimicrobial resistant surveillance both at local and national level. (117, 212)

9.6.1.3 Pseudomonas aeruginosa

There has been a tremendous increase in infections caused by multidrug resistant Gram negative bacteria especially *P. aeruginosa, K. pneumoniae* among others. For these organisms, treatment options become very limited, such that polymyxins are therefore the only available active antibiotics for *P. aeruginosa*. (213-217) In vitro colistin has shown excellent activity against Gram-negative bacilli including multidrug-resistant *P. aeruginosa*.

Our data seem to confirm this observation and showed that colistin resistance rate was 1.9% over the 5 years period of which data were available. This is consistent with several studies that reported low rates of colistin resistance.(139, 218-222) Despite adverse effects of

nephrotoxicity and neurotoxicity experienced by patients taking polymyxins which lead to its discontinuation in the 1970s, (214, 215, 223-225) treatment outcomes are good and it is now considered as the last alternative treatment of Gram-negative sepsis when other drugs such as extended spectrum cephalosporins, aminoglycosides and quinolones are found to be ineffective. (215, 225) Our data showed similar high rates of resistance as were previously documented by Pfaller et al. (113) to carbapenemes, extended spectrum cephalosporins as well as fluoroquinolones (Chapter 5, Table 5.3), which means that treatment options for such infections are minimal and quite challenging.

It is disheartening to learn from previous studies done in Canada, United Kingdomand India have reported high rates of colistin resistance ranging from 12% to as high as 50%.(226-231) This diminishing antimicrobial activity of colistin against Gram-negative pathogenic bacteria causing nosocomial infection is a clinical and public health concern due to a tremendous increase of multidrug-resistant strains in the absence of new antibacterial agents to treat such infections. Therefore, regular and timely monitoring of antimicrobial resistance patterns would play a crucial role in slowing down development of resistant strains among Gram-negative nosocomial pathogens.

9.6.2. Differential patterns of resistance by different age-groups

The study showed that the frequency of occurrence of nosocomial bacteraemia in the <5 years old population was high and rates of resistance to antimicrobials i.e. cefepime, oxacillin and amoxclav, among others, were proportionately higher among <5 years old and significantly different across age-groups (p=0.001). The proportion of MRSA was significantly different accross age groups and high among the <5 years olds. A similar pattern was observed for KP isolates where the proportion of ESBL-KP was significantly different

between age-groups (p=0.003) and 78.2% in the under five years old children. Braykov et al. in the USA (190), showed lower KP resistance rates to extend spectrum cephalosporins in the paediatric patients in general (i.e. age <18 years) ranging from 5.9%-8.3%, showing that the rates of resistance in South African hospitals might be higher than observed in developed countries.

Such pattern is not uncommon due to predilection of SA and Gram negative infection in the younger age-groups as a result of high colonization of SA bacteria on the skin and nasal area. Due to immature immune function, the under five children particularly infants, are prone to invasive bacterial infection from normal flora. As a result of frequent bacteraemia episodes, exposure to antimicrobials and selective pressure puts them at higher risk of carrying resistant isolates. (192) Such evidence should support improved clinical decision making in the empirical management of childhood cases of bacteraemia in public sector health services.

9.6.3. Gender differences in the pattern of resistance

There were more episodes of nosocomial bacteraemia among the male population compared to females, for all the three selected pathogens as highlighted in chapter 3, table 3.1 of section 3.3. Tiemersma et al. (147) reported from the European Antimicrobial Resistance Surveillance System, a higher frequency of MRSA isolates from men compared to women (21% versus 18%, p = <0.001) respectively. This may be an indication of a global pattern. It is however unclear why more males had a bacteraemia episode than females. We can only speculate that possible selective blood culturing could have played a role, or more males report to the hospital with more severe illnesses than females, hence warranting a blood culture investigation. This might speak to differences in health seeking behaviour between

males and females, with females being more health conscious thus reporting earlier for clinical assessment than males thereby ending up with empirical antimicrobial treatment.

In addition, we observed that females with KP bacteraemia were significantly more likely to have antibiotic resistant isolates compared with males. This is contrary to the findings of a study done by Braykov et al. (190) in the USA that looked at a large number of isolates that were collected over a decade (1999-2010). They found that isolates from male patients had significantly higher likelihood of antibiotic resistance amongst 3rd generation cephalosporins and carbapenem. Furthermore, we observed that males were significantly less likely to have MRSA than females. This might be due to the fact that the frequency of isolates was higher in males than females as similar to what was reported by Tiemersma et al. (147) We do not know the reason behind these findings, as such, further investigations are warranted.

9.6.4. Geographical differences in antimicrobial resistance (within country variation) by hospital and province

Our analysis revealed that there was a wide variation of antimicrobial resistance by hospital location and province. Antimicrobial resistance was higher generally in Gauteng Province hospitals as opposed to Western Cape Province hospitals. For example, making reference to the 2010-2011 study time, data showed that in Gauteng Province, amoxclav resistance (a surrogate marker for MRSA) was lowest at SBPAH (38.1%) and highest at CHB (83.6%) while in the Western Cape Province, MRSA was lowest at GSH (35.8%) and highest at TH (46.2%), while in the Free State Province, at UH resistance was 48.9%. Looking at this pattern, we can deduce that MRSA is generally higher in hospitals around Gauteng province as opposed to hospitals in Western Cape Province.

Stratified analysis by hospitals presented a more robust landscape for comparing distribution and patterns of antimicrobial resistance. The results from the retrospective data were similar to the July 2010- June 2011 prospective data, confirming a clear variation in resistance pattern by geographical location despite systematic error in the reporting of MRSA in Gauteng hospitals. We may however suggest that such variation could possibly be due to differences in health care services available, including differences in blood culturing practices among these hospitals.

Variation in clinical practices regarding blood culture specimen collection might bring differences in the spectrum of patients included for blood cultures which may differ between hospitals/provinces, e.g. in some hospitals/provinces only very critically ill patients might have had blood cultures done. We can assume that selective blood culturing might have influenced the pattern of resistance observed as the denominator on which to base the proportion or rate of resistance might be different depending on the volume of collected blood cultures.

The apparent decline in MRSA in Gauteng province is a unique finding and has not been reported before in South Africa. Occurrence of resistant bugs might also be due to climatic differences leading to differential occurrence of resistant bugs precipitated by multiple factors. Differences in prescribing habits between these hospitals might have led to differential antibiotic exposure level among patients to SA bactereamia.

9.6.5 Differences in laboratory operations and geographical variations in rates of antimicrobial resistance

In the course of conducting our study, we had to understand and bring a plausible explanation to answer the question "Would variation in laboratory methods explain the observed difference in rates of antimicrobial resistance?" Our data did not allow us to find a definitive explanation for the observed variation in rates of resistance between hospitals/provinces.

The 7 NHLS laboratories which were part of this study are all associated with academic institutions. We observed that the methodology for performing blood cultures were similar across sites (a combination of automation and manual methods), but the automated equipment that are used for the identification and evaluation of the susceptibility profiles of bacteria (i.e. Vitek 2 and MicroScan) produced comparable susceptibility results as well as organism identifications. (232)

All laboratories use similar MIC break-points as per the CLSI guidelines. (151) As alluded to in chapter 4, section 4.2, despite slight differences in the type of microbiology systems used, such systems were efficiently validated for susceptibility testing and organism identification and confirmed to produce comparable results. Therefore, we can conclude that laboratory operations might not explain the observed variation in results seen between different hospitals in different provinces.

9.6.6 Observed rate of MRSA

The rate of MRSA of 54.1% observed in the prospective analysis of blood culture data from Antimicrobial Resistance Surveillance and Research (ARSR) (Chapter 6, Figure 6.1), was found to be higher than what was observed in the systematic review (33.0%) (31) of

published data from South Africa over a period of 12 years (2000-2011) but higher than 15.4% (Chapter 5, Table 5.3) that was observed from the retrospective data over a 5 year period (2005-2009).

The rates of MRSA for Gauteng hospitals (0.4-4.5%) were much lower than would be expected when compared to baseline data from the systematic review. On the other hand, when compared to rates for the Western Cape hospitals and Free State (30.4-43.0%), these rates appear closer to the estimated MRSA rates as observed from the systematic review, suggesting that MRSA might not be <30% for the Gauteng hospitals (Chapter 5, Table 5.3). The reason for this observation remains uncertain however it might be that antibiotic susceptibility results documented from Gauteng hospitals were systematically underreported.

This characteristic was observed for SA susceptibility testing done in hospitals around Gauteng Province. Why only in Gauteng hospitals and only for MRSA and not other antibiotics remains unclear and requires further investigation. Laboratory methodology does not seem to explain this anomaly, due to the fact that systematic observation of laboratory methods carried out in all hospitals participating in the study, revealed that all laboratories used similar methods of blood culturing and susceptibility testing.

Even though NHLS laboratories used different automated microbiology systems for susceptibility testing i.e. Tygerberg hospital used Vitek 2 and CMJAH used MicroScan, these automated microbiology systems have shown to produce comparable results (123), after being appropriately validated by the NHLS. The NHLS break-points used to assess

susceptibility level for various antibiotics are according to the CLSI guidelines, (151) which means that there is a standardised method of susceptibility testing that is followed across the spectrum of the NHLS operations in the country.

In view of this, susceptibility data can be comparable across sites in South Africa. In testing MRSA, various laboratories across these sites used either oxacillin or cefoxitin disks (151) to assess presence of MRSA among SA isolates from cultured blood. Use of either disk did not produce different susceptibility patterns for SA resistance. This made comparability of MRSA across the study sites scientifically acceptable.

9.6.7 Comparability of laboratory methods for blood culture and susceptibility testing between two different geographical locations

Our study found that laboratory methods for blood culturing and antimicrobial susceptibility testing that were followed by the diagnostic microbiology laboratory at Muhimbili National Hospital, Dar es Salaam in Tanzania were similar to those followed by NHLS microbiology laboratories in South Africa. In Tanzania, blood culturing was not a routine laboratory investigation; the test is ordered by clinicians in special circumstances such as persistence of clinical symptoms suggestive of bacterial infection after initiating patients on empirical antimicrobial treatment. The choice of the antibiotics is guided by local knowledge of the epidemiology of common bacterial pathogens in the area.

Observation of continued or worsening of clinical symptoms indicative of invasive bacterial infections spurs the clinicians to order a blood culture test, in order to identify the offending bacterial pathogen and assess susceptibility of available antibiotics to guide clinical

management of treatment failed patients. The challenge of such an approach is that since patients would already have been exposed to antimicrobials, the probability of a positive blood culture outcome becomes minimized. As such a majority of blood cultures end up being negative and are recorded as no growth.

The reason being that pre-exposure to antimicrobials may have led to suppressed bacterial activity which reduces the viability of bacterial pathogens. (233) This might have led to an underestimation of the actual burden of invasive bacterial infections, enhanced resistance development as bacterial pathogens were exposed to antimicrobial agents which they are not sensitive to, and creates an unwanted clinical situation due to challenges in effective management of patients who have masked clinical symptoms and negative blood culture results. (233) This status is a common occurrence in less developed and low income countries due to limited resources to perform blood cultures.

The situation was somewhat different from that of South Africa as the volumes of blood cultures done generally looked high due to non restriction of blood culture tests. In most cases in South Africa, patients would access a blood culture test before being prescribed an antibiotic. Once the index of suspicion for bacteraemia is high, requests for blood culture tests are done routinely. It was observed that multiple blood cultures are done, causing an influx of duplicate blood cultures results (Chapter 2, Table 2.2), which if overlooked during analysis of blood culture data, may lead to an overt overestimation of the burden of bacteraemia in the population as a direct consequence of multiple blood cultures.

The laboratory at Muhimbili hospital looked greatly under resourced in both personnel and equipment. At the time the laboratory audit was being conducted, automated blood culturing

machines had not been functioning for over 6 months, meaning that the laboratory relied only on manual processing of blood culture specimens including antimicrobial susceptibility testing. This might cause a strain on the limited human resources available. However, since the blood culturing is selective, the volume was quite low compared to what was observed in the microbiology laboratories in South Africa. It must be noted that manual methods have been the traditional way of blood culturing over many years and have shown comparable results with automated machines (234) hence are not inferior to the automated methods.

In addition to this, all microbiology laboratories i.e. NHLS laboratories and the laboratory at Muhimbili hospital use CLSI guidelines for MIC break points in the conduct of susceptibility testing, (151) which means that susceptibility test results between these laboratories are expected to show similar results. For this reason, we may conclude that the observed differences in the processing of blood cultures may not produce variation in the patterns of resistance among the tested antimicrobials other than that which is inherent. Since these are geographical distinct areas, the local epidemiology of bacterial pathogens and exposure to antibiotics might be different.

Differences in racial composition, socio-economic status, antimicrobial prescribing, infection control adherence, duration in hospital stay, patterns of hospital admission, laboratory methodology with appropriate quality control practices and recording systems may all affect bacterial resistance patterns. Therefore, susceptibility data produced by laboratories from these two geographically distinct areas with different resources and population structure could produce comparable findings provided the methodologies are standardized according to international norms and laboratories employing appropriate quality assurance practices.

However, sampling between hospitals based on clinicians' decisions on when to perform blood cultures is likely to differ.

9.6.8 Comparability of blood culture data and antimicrobial resistance patterns between CDW and GERMS-SA databases

A systematic evaluation of the two databases revealed wide variations in SA and KP isolates, which were on the CDW database but were not found on the GERMS-SA database, which we defined as cases in the audit procedure. This has major implications when it comes to estimating the burden of SA and KP bacteraemia reported in various hospitals across the study sites. For example, Chapter 7, Table 7.1, shows the number and proportion of cases which were missing in the GERMS-SA database from 1st January - 31st December 2011, by hospital location, specifically looking at SA and KP isolates. In total, 409 SA and 365 KP isolates were identified as missing in the GERMS-SA database during the audit process. High proportions of missing isolates were from hospitals in Gauteng Province, predominantly from CMJAH, contributing almost 30% of SA and 36% of KP isolates respectively. The lowest proportions of missing isolates were from Groote Schuur hospital in the Western Cape Province, contributing only 5.6% of SA and 0.3% of KP isolates.

All cases were identified and tested at the NHLS laboratory and then shipped to GERMS-SA and added to its data base so as to improve its completeness of the number of identified isolates. It remains unclear why hospitals such as CMJAH had a higher percentage of missing isolates on the GERMS-SA database while GSH had only few missing cases. Since GERMS-SA isolates require shipment, GERMS-SA based surveillance is more labour-intensive and it is possible that some hospitals shunned this extra effort. We therefore assume that differences in the rigour regarding collection and reporting of isolates under surveillance program might

be a contributing factor to missing data. This might have been due to, among other reasons: lack of understanding of the value of the surveillance regarding these organisms; lack of enthusiasm or diligence among the laboratory staff to report these organisms; negligible level of awareness regarding the need to collect and report such isolates, lack of human resource leading to high work load among laboratory technologists dealing with blood culture procedures leaving no room for them to focus on reporting isolates of SA and KP to the ARSR.

These might be among the most plausible reasons why there is a difference in the number of isolates in the GERMS-SA database, which draws its samples from the same source as that of the CDW database. In this case GERMS-SA data might not have been a proper gold standard for determining incidence but could assess reliability of blood culture data drawn from the CDW database. In addition, since both databases generate their data from the same source, which is the laboratory request form which was completed at the hospital where the blood cultures were done, missing data in terms of age, gender, and other demographic parameters remained the same in both situations.

This means, missing data on demographic parameters on the CDW database will be the same as on the GERMS –SA database as the surveillance does not particularly collect data from the point of blood culture collection but from the laboratory after blood culture procedures have been completed. Therefore, as data were being analysed and compared, we expected that there will be no differences in completeness of data for demographic parameters between CDW and GERMS-SA with the exception of Gauteng MRSA data that showed there was a systematic error in reporting. The reason being ARSR receives clinical isolates from the same

microbiology laboratories. As a result incomplete data on laboratory request forms reflect the same missing data in the ARSR program at the NICD. Hence using ARSR as gold standard comparator for demographic data such as age and gender was not suitable and was therefore not taken further.

9.6.9 Quality of antimicrobial resistance data of SA and KP: CDW versus GERMS-SA

When assessing the quality of resistance data denoted in the database as antimicrobial susceptibility, it is evident that the quality of susceptibility data drawn from the prospective study, i.e. for the period July 2010 to June 2011, was good. No missing data on susceptibility test results were seen during cleaning and coding of the data. All antibiotics tested intrinsically or as part of the requirement for the surveillance or quality assurance were available.

Most of the antibiotics were tested against the same number of isolates, which means that the denominator remained constant when assessing resistance rates of different antibiotics. This meant that more precise estimates of resistance were generated from this data as opposed to the retrospective data of 2005-2009, which had a lot of missing data on antimicrobial susceptibility test results. Therefore, many other antibiotics were not assessed for resistance as there was a sign of incompleteness.

In view of this, we can deduce that data collected prospectively might have provided a clearer picture of the resistance pattern. However, despite challenges in completeness of resistance data, rates of resistance to antibiotics of clinical relevance generated from GERMS-SA,

ARSR database and CDW database with exception of Gauteng MRSA, data were quite comparable (Chapter 7, Tables 7.2 and 7.3). This is an important observation as it signifies that routine blood culture data source could sufficiently be used for antimicrobial resistance surveillance after considering areas requiring improvement, i.e. point of data correction in clinical departments i.e. wards, casualty, intensive care unit etc., as completion of laboratory request forms are done there. Once information is omitted from the laboratory request form, such omission does not get resolved at the laboratory level.

9.6.10 Active laboratory based invasive pneumococcal disease surveillance: A model surveillance system

The invasive pneumococcal disease (IPD) surveillance carried out nationally by GERMS-SA can be highlighted as a model of an effective surveillance system (Appendix 12.2)**.** Using data through this surveillance, we were able to show through a systematic analysis using multivariate logistic regression modelling that age, younger than 1 year, Pitt Bacteraemia Score ≥4 and HIV infection were independent risk factors for death in children with meningitis, and notably that malnutrition increased the risk of death among children with other IPD.

This data added to the body of knowledge regarding identification of population at risk for death that needs enhancement of targeted preventive health services, including among others a catch up program for pneumococcal conjugate vaccine for immunization of children particularly in HIV high burden areas, so as to reduce incidence and excess mortality in the risk children. This fulfils the aim of a surveillance system, which is to collect, collate, analyse, interpret, make data available and apply such information to the control of communicable diseases. (63, 235)

In spite of the fact that this was a laboratory based surveillance such as that presented in preceding chapters for antimicrobial resistance, the distinct difference is that IPD surveillance data includes clinical parameters as well as data on outcome and therefore, we were able to predict treatment outcomes using such surveillance data as highlighted earlier. This information is gathered by surveillance officers as part of an active surveillance program. Presence of clinical information adds value to the surveillance data as information obtained can be used to formulate strategies on how disease can effectively be controlled.

That is the main challenge of antimicrobial susceptibility data, as the only information we can come up with is patterns of resistance, but we have no idea if such resistance has a direct impact on patient survival. We are also not able to find out if certain clinical conditions such as HIV are potential drivers of resistance, since we do not have such data available. (148)

9.7 Potential Study Biases

9.7.1 Completeness of antimicrobial resistance data

The study used data extracted from the CDW which showed a reasonable degree of missing parameters on demographic factors and susceptibility test results. This created a window of opportunity for information bias to introduce potential underestimation of the actual magnitude of antimicrobial resistance over the given study period would occur. It can be assumed that particular antibiotics which might have been tested and had missing data, might not display actual patterns and rates of resistance due to insufficient data. We therefore did not further analyse such data for rates of resistance amongst antibiotics if less than 20 isolates were tested for antimicrobial susceptibility.

It should also be noted that, blood culture data transmitted via the file transfer protocol (FTP) from all NHLS laboratories to the central data repository, had an inherent weakness of data loss due to among other reasons, the slowing down or breakdown of internet service or as a result of manual entry of laboratory results without a back up of double entry or validation system of data entry. Comparing retrospective (routine activity) and prospective data (in a research environment), we observed a systematic difference in the quality of data which might have arisen due to differences in rigourousness in maintaining data quality, or strictness in conducting susceptibility tests, or procedures for data entry of blood culture test results as well as an active verification blood culture of data. Prospective blood culture data were more complete hence of better quality with regard to antimicrobial susceptibility test results compared to retrospective data.

9.7.2 Underestimation of antimicrobial resistance rates

Invasive bacteraemia cases captured and reported by the routine system (CDW) and GERMS-SA surveillance system might not be representative of all the cases of invasive SA, KP and PA bacteraemia occurring in South Africa over the study period. This might be because of differences in blood culture taking practices between the different hospitals or due to selective blood culturing.

It is not a common practice to do blood cultures in all febrile illnesses that present to a clinic or hospital; hence some patients who genuinely had bacteraemia caused by any of the selected organisms might have been missed. Patients who were sick and self medicated themselves or died at home due to a febrile illness or in transit to a clinic or hospital, might also not have been captured by the surveillance system. Therefore, the incidence of bacteraemia and antimicrobial resistance rates reported earlier is certainly an underestimation

of the true magnitude of bacteraemia and associated antimicrobial resistance due to these selected organisms. Thus surveillance bias might likely have existed in this study. (63)

9.7.3 Bias in analysis of associated risk factors

Underestimation of disease burden might have introduced an erroneous estimation of rate of resistance and associated factors in our study. Even though, missing data are a common problem with routine or passive surveillance system, the coverage of our sample population was quite broad. We analysed all reported isolates over the study period from the seven major hospitals in South Africa.

Since there are no major differences observed in laboratory methods between various NHLS clinical microbiology laboratories, we can deduce that susceptibility data from these areas is quite comparable, except for Gauteng MRSA data that showed there was a systematic error in reporting. Use of strict case definitions for antibiotics to be included in the risk factor analysis (resistance to at least 1 antibiotic for retrospective data) might have helped to minimize such form of bias. To minimize bias in risk factor analysis for prospective data, we restricted our analysis to isolates that showed MRSA and ESBL on susceptibility testing.

9.8 Potential residual confounding factors

In assessing factors associated with antimicrobial resistance, we, initially, examined all factors in a univariate model to find out if they were associated with increased likelihood of resistance. Significant factors at P≤0.1 were included in the multivariate logistic regression model where each factor controlled for the confounding effect of the other. This method helped to control for the confounding effect of several factors at the same time. Factors

significant at P≤0.05 were then considered independent significant factors associated with antimicrobial resistance. However, residual confounding factors affecting the association between the exposure factors identified and resistance remains a possibility.

In this study, due to lack of data, we did not examine all factors that could potentially be associated with resistance such as pre antibiotic exposure before accessing a blood culture test. However, our findings seem to suggest that the association observed between factors associated with resistance might not have been due to chance, random error or residual confounding. (63)

9.9 Generalizability

Blood culture data used in this study originated from tertiary public hospitals all of which were associated with academic institutions. There might be relative differences in terms of operations and access to laboratory services between academic and non-academic hospitals such as those from rural areas. This might limit generalizability of our study findings beyond the populations from which data originated.

In addition the difference in the distribution of isolates by province and hospital location as observed, might also limit generalizability of our study results to other provinces and hospitals situated in more rural areas. In the light of the foregoing facts, our findings might only be extrapolated to the defined area where the study was done.

9.10 Study Strengths

- Our study used data over a 6 year period from tertiary academic hospitals in three of the densely populated provinces in South Africa to evaluate trends and patterns of resistance and determine factors associated with antimicrobial resistance amongst SA, KP and PA bacteraemia. The coverage was wide and over a longer period of time, unlike most studies done are usually local and over a short time interval. (26, 99)

- The large numbers of isolates that were included in our study enabled us to detect significant differences in risk factors for antimicrobial resistance amongst the three pathogens that were investigated.

- Systematic comparison of resistance patterns between retrospective data aggregated routinely and prospective susceptibility data (with well designed quality assurance procedures) adds a great value to this study. Such an undertaking has not been carried out before and exposes the value of routine data and its ability to be used to monitor patterns and trends of resistance in South Africa, of course with some improvement to be done.

9.11 Study Limitations

- The study used laboratory data which, unfortunately, did not have details on clinical parameters including treatment outcomes (i.e. death, severity of illness etc.). We were therefore, not able to make an in-depth analysis to assess the association between resistance and clinical outcomes.

- Our study involved retrospective data analysis using CDW and GERMS-SA surveillance database. The data we analysed was not collected with our research questions in mind as such, some parameters which would have been of interest to us,

such as date of admission which would have helped to determine nosocomial infection, were not collected.

- We did not have complete data on all antibiotics tested particularly from the CDW repository, which was critical to this study; as such we may have potentially underestimated the magnitude of nosocomial bacteraemia and that of antimicrobial resistance. To minimize this we restricted our analysis to include only antibiotics that had complete data on susceptibility test results.

- This was an analysis using an existing dataset hence other than knowing the antibiotic that were tested, we did not have information on the antibiotics that were prescribed for patients from whom the isolates were identified, neither dosage nor duration of treatment that was given, and not even the outcome of the patients. We were thus unable to assess association between resistance and antibiotic treatment prescribed during the episode of bacteraemia.

- Data for antibiotic susceptibility for year 2005 was available only from the second half of the year. This might have made comparison of resistance by year a bit obscure for the year 2005, despite the fact that similar proportion of isolates were observed for the three pathogens in that year.

- Blood culture data from CDW and GERMS-SA databases shared a common weakness which was that, demographic data are completed on the laboratory request form by the clinical departments such as admission wards, casualty etc. which are the point of blood culture collection. Once an isolate is identified, the laboratory sends isolates to the ARSR, completes a GERMS-SA surveillance form using data from the original laboratory request form. This means that all missing data on the laboratory request forms in the wards are then passed on to the GERMS-SA database at the

ARSR making GERMS-SA data an effective gold comparator against the CDW data except for susceptibility data.

- We might have underestimated the overall antimicrobial resistance rates for SA, KP, PA due to use of blood samples only excluding other samples i.e puss swabs, urine, stool, sputum etc.

9.12 Suggestions for Improvement

In view of the findings discussed in this chapter, there is need to enhance the laboratory information system as well as laboratory operations in order to improve the quality of antimicrobial susceptibility data. In doing so, precise estimates of patterns and trends of antimicrobial resistance will be determined. To enable this to be achieved, the following needs to be considered:

- Completeness of demographic data such as age and gender at the point of clinical request of and collection of a blood culture in the clinical departments. This requires proper completion of a laboratory request form and labelling of the blood culture bottles.

- Complete and appropriate entry into the DISALab LIS of data of antimicrobial susceptibility results in the microbiology laboratory.

- Relegation of the policy to suppress antimicrobial susceptibility results by pathologists in the microbiology laboratory. Such a practice, evident in the Gauteng hospitals, gives erroneous picture of the state of antimicrobial resistance particularly amongst antibiotics that were suppressed hence their data were not seen on DISALab LIS, for example the pattern of MRSA in Gauteng Province detailed in Chapter 5, Table 5.3).

- Maintain regular quality control of blood culture data to assess reliability of susceptibility results and identify if the microbiology laboratories produce comparable results to other well established laboratories such as the CDC.

- The NHLS should fast track the implementation of Trackcare LIS, a system that operates in real time, as this will provide timely data. Such a system would improve timeliness on delivery and dissemination of patterns of antimicrobial resistance to clinicians and public health practitioners.

- The active surveillance of antimicrobial resistance (ARSR) which is part of the GERMS-SA should enhance its operations by including clinical data on their data collection form. This among others should incorporate data on duration the patient stayed in hospital before blood culture was taken; name of antibiotics taken before a blood culture was done; clinical syndrome as well as outcome of the illness on discharge (i.e. discharged alive, dead); acute physiology, age, chronic health evaluation (APACHE III) score etc. for patients admitted in intensive care unit. (236)

CHAPTER 10   Conclusions and Recommendations

This chapter provides some conclusive detail on the status of antimicrobial resistance in South Africa with a focus on the seven sites that were part of the study. Here, we highlight the main findings and bring in the public health implications, suggestions for improvement as well as perspectives for future research.

10.1 Conclusions

The patterns of antimicrobial resistance are varied, high rates of resistance against a wide spectrum of antibiotics were observed among the selected pathogens. Multi drug-resistant strains of MRSA seem to be rapidly increasing, including the more serious vancomycin resistant strains of SA seen in some hospitals in Gauteng Province. An increase in bactereamic episodes due to glycopeptides non-susceptible SA strains will be predictably detrimental in managing MRSA. This is because clinicians managing such patients will be left with very few therapeutic options to choose from or no options for effective treatment of the patient. This calls for action to enhance laboratory procedures and optimum practices in order to detect MRSA on time including accurate and ongoing identification of glycopeptides resistant strains.

In addition, regular, active, and standardized surveillance of MRSA across sites to provide timely data on the antibiotic susceptibility pattern for implementation of effective hospital infection control programs need to be emphasized. Furthermore, such data will also aid the formulation of antibiotic prescription policy to guide empiric treatment and avoid misuse of

the valuable antibiotics. Studies to monitor the epidemiology of MRSA using molecular techniques in these hospitals are highly recommended.

The frequency of ESBL as well as carbapenems-resistant *K. pneumoniae* is on the rise as evidenced by our data which supports global trends.(190) Similarly our data supports previous reports of emerging resistance amongst polymyxins (Chapter 5, section 5.3.10), which are the last line antibiotics in treating multi-drug resistant *P. aeruginosa*. (214, 215, 225) Carbapenems are reserved drugs in the clinical management of *K. pneumoniae* bactereamia and therefore effective intervention to curb the growing crisis of carbapenems-resistance should be reinforced. (134, 206, 207)

It is therefore imperative to reinforce ongoing surveillance of invasive bacterial infections, paying special attention to the resistance profile of individual pathogens both at a local, national and regional level. Taking cognizance that resistance varies by geographical area including by different hospitals (237) it is important that regular assessment of local resistance data be enhanced as that would guide choice of antibiotic treatment by physicians in managing patients with multi-drug resistant nosocomial bacteraemia.

10.2 Public Health Implications

The study found emerging vancomycin resistant *S. Aureus* (VRSA), carbapenem-resistant *K. pneumoniae* (CRKP) and colistin resistant *P. aeruginosa* in South African tertiary public hospitals. In addition, geographic variation, gender and age differences in resistance were clearly demonstrated. These findings, as outlined above, are valid and unlikely to be due to chance, sampling bias or residual confounding. Age, gender and geographic variations in

antimicrobial resistance in patients with invasive nosocomial infection in South Africa have not been examined before at such a large scale since good quality data on laboratory-confirmed invasive nosocomial infections were lacking. To our knowledge, the utilisation of LIS as a tool for monitoring antimicrobial resistance surveillance among nosocomial bactereamia has not been previously assessed in South Africa. In addition, assessment of factors associated with resistance using multivariate logistic regression models has also not been done before.

Furthermore, this was the first time to conduct a comparative assessment of resistance data comparing resistance patterns found through a systematic review, retrospective and prospective analysis for the purpose of validating the reliability of routine blood culture data from the NHLS microbiology laboratories that aggregates at a central repository. Most studies done in South Africa have focused on analysis of small scale data either from a single hospital or simply presenting frequency of resistance for selected antibiotics. (26, 99, 123) We believe our study is robust and provided solid evidence on the reliability and utility of LIS as an effective tool for surveillance of antimicrobial resistance.

We believe that our study is unique and provides information to individuals in clinical and public health practice to understand the challenges regarding patient management if faced with few treatment options due to increasing resistance and to understand the population with a high burden as well as at risk of carrying resistant bugs. Clinicians should be aware that young children particularly under the age of 5 years have a higher burden of nosocomial bacteraemia and they also have higher rates of antimicrobial resistance.

Therefore, this group of patients carrying the greatest risk of resistance needs to be managed effectively to minimize adverse outcomes such as prolonged hospitalisation and death. Hospital infection control epidemiologists and public health practitioners should therefore reinforce targeted preventive health services to control nosocomial bacteraemia in this age group.

The findings from this study have broader implications on public health policy, to slow down the development of resistance to antibiotics that are key to effective management of bacteraemia. In addition, the study advocates for effective commitment to hospital infection prevention to reduce the burden of nosocomial infections as there are no new drugs to treat multi-drug resistant bugs.

10.3 Recommendations

In view of the foregoing, the following recommendations are made:

- Active surveillance of antimicrobial resistance that is carried out by GERMS-SA should incorporate clinical data so as to use such data to find the association between resistance and clinical outcomes such as mortality.

- More focused testing of antibiotic susceptibility, aiming at assessing resistance among antibiotics in regular use. Laboratories should minimize intrinsic testing, such action will improve the quality of data as the DISALab will only handle data for few antibiotics tested other than a large battery of antibiotics. Focussed testing should target the following antibiotics: *S.aureus:* ampicillin, cloxacillin, vancomycin, gentamicin, erythromycin, linezolid, clindamycin, rifampicin, fusidic acid, cotrimoxazole; *K.pneumoniae*: ampicillin, amoxycillin-clavulanate, imipinem, ertapenem, cefazolin, ceftazidime, cefuroxime, cefoxitin, cefotaxime, ceftriaxone, cefepime, piperacillin-

tazobactam, gentamicin, amikacin, ciplofloxacin, nalidixic-acid, colistin, cotrimoxazole; *P.aeruginosa:* imipinem, meropenem, ceftazidime, cefepime, piperacillin-tazobactam, tobramycin, amikacin, ciprofloxacin, colistin.

- Enhanced regular monitoring of patterns of antibiotic resistance in hospitals as data from such system would guide formulation of standard treatment guidelines to be based on local and objective data. In addition data required for efficient linkage of the frequency of resistance to prescribing must be collected within the antimicrobial resistance surveillance system.

- Antimicrobial prescription policy should be developed based on local patterns of resistance to guide antimicrobial prescriptions in hospitals; hence the national treatment guidelines should be used for purposes of reference only.

- Regular in-house training of both ward and laboratory staff regarding the value of antimicrobial resistance surveillance and how such is affected by the quality of data captured.

10.4 Suggestions for further studies

In view of the findings discussed in this chapter, further research is needed to gain a clearer understanding regarding the geographical differences in the patterns of antimicrobial resistance among SA, KP, and PA. In addition future studies should investigate:

- The association between antibiotic resistance and mortality in a South African context. This should focus on assessing clinical outcomes of patients who received an antibiotic that is resistant to the isolated bug.

- The influence of gender on antimicrobial resistance since the study showed that a higher proportion of resistant isolates were found in men, but also that male gender

was associated with increased risk of antimicrobial resistance as shown in both retrospective and prospective data analysis.

- The association between antibiotic resistance and antibiotic use, which will involve linking data on antibiotic resistance and antibiotic use.

- The effect of discordant therapy on treatment outcome, which will involve relating antibiotics prescribed in hospitals versus antibiotics tested for resistance and clinical outcome.

- The prevalence of both community and hospital acquired MRSA in South Africa (i.e. MRSA identified on admission versus MRSA identified ≥2 days after admission).

10.5 Contribution of this work to the field of research in antimicrobial resistance

This research has clearly demonstrated that antimicrobial resistance is high in South Africa. Of interest is the significant variation of antimicrobial resistance by geographical location, gender and age, with children <5 years being more at risk of carrying a resistant strain. Lastly, we have also shown the value of the LIS as an essential tool for public health surveillance of antimicrobial resistance in spite of its inherent weakness of incompleteness of susceptibility data.

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12.0 Appendices

Appendix 12.1: A narrative Review of the Laboratory Information System and Its role in Antimicrobial Resistance Surveillance in South Africa

Scientific Research

A Narrative Review of the Laboratory Information System and Its Role in Antimicrobial Resistance Surveillance in South Africa

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Abstract

A laboratory information system (LIS) established in a microbiology department has the potential to play an important role in the quality of microbiology data such as culture of blood, urine, stool, pus swab samples etc. Such data could be effectively utilised to measure the burden of antimicrobial resistance among patients presented to various hospitals and clinics with an episode of an infectious illness of bacterial origin. A variety of clinical and epidemiological investigations are conducted using culture data and the presence of an electronic system such as LIS enhances such investigations and improves the reliability of measures of antimicrobial resistance owing to improved data quality as well as completeness of data gathered as opposed to paper based system. Therefore to improve surveillance of antimicrobial resistance in South Africa, there is a need to reinforce the functionality of the LIS in both public and private microbiology laboratories as this will help to improve internal quality control methodologies.

Keywords

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1. Introduction

The laboratory information system (LIS) is a data processing and dissemination technique used in the laboratory to deliver accurate and understandable results within a reasonable timescale as requested by clinicians. The system entails a sequence of events which include, delivery of samples to the laboratory, sample accessioning, analysing, verifying and approving results or reanalysing samples and releasing results to the clinicians who requested the tests [1]. In short, the concept of LIS refers to the computerisation of the laboratory system or automation of clerical labour-intensive activities associated with the processing of laboratory results to improve accuracy and turnaround time of results. Automation of laboratory activities removes the element of manual reporting, increases productivity and allows access to retrospective data for analysis [1]. Previous studies have reported an improvement in the accuracy of data and turnaround time of laboratory results after installation of the LIS [1].

The increase in clinical specimens as well as the consolidation and integration of laboratories and tests have resulted in the generation of significant volumes of tests and data. The complex and large quantity of data that these laboratories accumulate as well as the continued demand for data to support public health surveillance for effective disease prevention has resulted in the need for operational LIS or Laboratory Information Management System (LIMS). Although these terms are often used interchangeably, LIS typically refers to strictly clinical operations and LIMS refers to all others including Public Health, Pharmaceutical, Research and Development, Manufacturing, Food & Beverage, Forensics, Chemicals and other fields as well. For the purpose of this paper we will use the term LIS. This system can efficiently integrate and handle all sophisticated processes and procedures related to data from different laboratory departments. These include microbiology, parasitology, virology, histopathology, biochemistry, hematology, endocrinology, cytology, toxicology, serology and immunology [1] [2]. Data for all such activities for laboratory services aggregates at the data warehouse repositories. This data is available to enhance business (*i.e.* billing of laboratory test), research and training aspects of the organisation.

2. General Description of LIS Components and Function

A laboratory information system is illustrated in **Figure 1**. The system is composed of the following components: hardware and network connectivity (computer system), LIS/LIMS software (computer programs), human capital (physicians who order laboratory tests, transport samples, etc.), trained laboratory staff who setup laboratory tests, execute procedures (*i.e.* blood culturing, HIV testing, susceptibility testing etc.) data analysis, quality control and reporting (laboratory results) **(Figure 1)**.
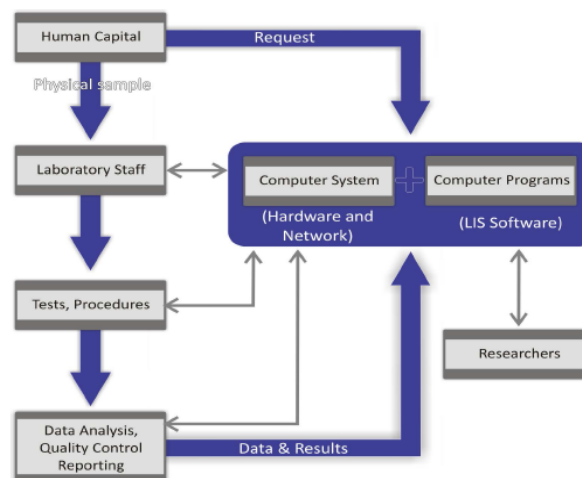

Figure 1. Flow chart demonstrating the components of a laboratory information management system.

All components are integrated and support each other interactively in the collection, capture, processing, storage and the distribution of data obtained during analysis. The system puts the required efficiency of tracking and sorting laboratory data, improves turnaround time of laboratory results and allows for the retrospective analysis of data for surveillance or research purposes [3]. **Figure 2** describes the processes and procedures in the function of a LIS [4].
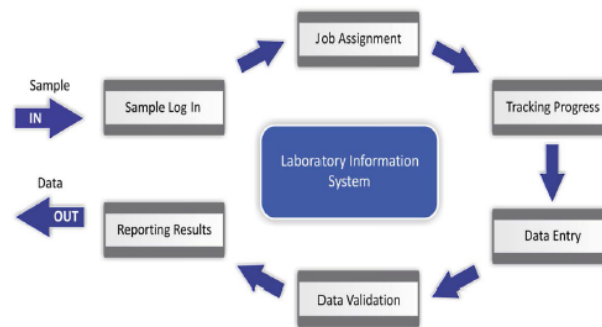

Figure 2. Laboratory information management system processes and procedures [4]. Adapted from Zhu, J. Indiana University, 2005. http://hdl.handle.net/1805/323

Although the LIS is a complex system, it works to simplify and improve the efficiency of laboratory operations, minimise data entry and other transcription errors as well as deliver valid and reliable laboratory results to the client in the most efficient way. In addition, the system provides a platform for surveillance such as monitoring antimicrobial resistance following the utilization of retrospective data archived in the LIS database [3] [5]. In South Africa, for example, the National Health Laboratory Services (NHLS) [2] a network of public health laboratories with a national footprint utilizes LIS software known as DISAlab that was supplied and maintained by the Laboratory System Technologies (Pty) Limited for the past two decades. However the DISA LIS has been phased out and replaced by the TrakCare LIS software developed by the Health Systems Technology [5] [6]. The LIS as a way of internal quality control measures allows for validation of data entries to detect errors as the system has built in check codes. In the event of failure, verification of data entered is then done manually by an alternative laboratory staff member. In addition, an external quality control process exists in microbiology laboratories. The accreditation or quality assurance process of each laboratory allows for improvement in the quality and reliability of data produced [7]. An acceptable margin of error of 5% indicating observational error in reporting quantitative measures should be taken into account when analysing routine microbiology data due to missing data which is an inherent weakness of the routine passive surveillance system. Some of the parameters entered into the LIS database are the following: Sample identification, time collected, time registered; demographics such as date of birth or estimated age, gender, hospital, ward, province, clinical diagnosis (scanty data),organism cultured, drug sensitivity (resistant/sensitive (R/S); minimum inhibitory concentration (MIC)), date sample was first registered and tested, date and time results were reviewed, instrument used for testing, sensitivity of antimicrobials, date clinician printed the results as well as first laboratory where specimen was taken. In South Africa, data from microbiology laboratories are routed to a central server the corporate data warehouse (CDW) [8] a data repository that is interlinked to the LIS. This includes data from cultures of blood, cerebral spinal fluid, stool and urine, pus swabs among others. Such routinely collected data could be used to monitor patterns and trends of antimicrobial resistance. The CDW composition [8] however provides limited platform for epidemiological investigations that aims to investigate specific clinical outcomes associated with antimicrobial resistance. The reason being that laboratory data originating from blood, urine, pus swabs etc., does not often contain clinical parameters such as severity of illness, outcome of treatment etc. This would henceforth make investigations linking clinical outcomes to antimicrobial resistance uncertain to undertake.

3. Variation in LIS

There are major challenges relating to the NHLS LIS which might originate from wide variation in the opera-

tions of the LIS between different laboratories. These emanate from wide variations in procedures that are used for gathering and reporting of culture and sensitivity data. Some of the underlying causes of such wide variability might be: 1) different reporting styles between different laboratories including different names used by different instruments; 2) instruments used vary between different laboratories *i.e.* other laboratories use more advanced instruments than others; 3) lack of standardisation across different laboratories which might affect scope of data generated.

4. Data Security System

The LIS data is password protected and each of the local laboratories has an electronic gate keeper, to monitor and minimise data corruption, and to access the data for research use (https://labresults.nhls.ac.za/). Since data originates from varying sources, the potential for data corruption is high hence appropriate security measures for access and utilisation are essential.

5. Data Quality

For an effective surveillance system to be sustained, data quality (*i.e.* completeness of demographics e.g. age, gender, geographical location; antibiotic susceptibility tests (*i.e.* MIC), resistant/sensitivity etc.) needs to have a primary focus. For this reason, it is paramount to monitor specific areas of the system where problems with data quality could be identified and appropriate intervention undertaken to improve data quality. To achieve this, the following issues need to be considered: 1) whether a researcher would accept at face value what was extracted from the database using the designed query; and 2) whether a researcher would be able to make any request for specific, logical, clear and unambiguous data elements in the query design. To improve the quality of data aggregated at the CDW [8], there is need for various players from the different microbiology laboratories to work collaboratively so as to minimise major variability of antimicrobial resistance patterns that might be originating to a large extent from data entry errors. Regular quality control exercises are essential to improve accuracy and reliability of culture data. To improve surveillance of antimicrobial resistance, in public sector hospitals the NHLS should introduce instruments that could be utilized effectively to generate reliable data elements to be used for various epidemiological investigations as well as aid clinical decision making regarding bacteraemia episode by clinicians in the clinical departments [9].

6. Conclusion

The LIS was not primarily designed as a research or surveillance tool. Its function has been to generate data accessible to the requesting client that could be used for appropriate and accurate billing of all tests done in the laboratory. Data has also been used to understand the volume of tests done, the time it takes to get results back to the patient and to plan efficient service delivery of the NHLS. We believe that such a system can be used as an effective surveillance tool to monitor development of antimicrobial resistance to various significant bacterial pathogens in our population since the process of acquiring culture and sensitivity data is inherently ongoing. Therefore, understanding the challenges of the system and suggesting ways of improving the overall system performance would be a step in the right direction for a well-established and functioning antimicrobial resistance surveillance program. Such a program would enhance our ability to contain the growing crisis of antimicrobial resistance that threaten our ability to treat patients effectively in South Africa.

7. Acknowledgments

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Appendix 12.2: Understanding laboratory methods and their impact on antimicrobial resistance surveillance, at Muhimbili national hospital, Dar es Salaam, Tanzania.

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Understanding Laboratory Methods and Their Impact on Antimicrobial Resistance Surveillance, at Muhimbili National Hospital, Dar es Salaam, Tanzania

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ABSTRACT

The study sought to describe laboratory methods and blood culture procedures and their impact on antimicrobial resistance surveillance among nosocomial bacteria. We conducted a systematic audit of blood culture procedures and practices in the Department of Microbiology, Central Pathology Laboratory at Muhimbili National Hospital, between 19th and 23rd March 2012. A total of 25 - 30 blood culture specimens were received each day as an indication of low volumes of blood culturing at this site. More blood culture requests came from the neonatal unit of the hospital, and were performed manually with high culture negative specimens. The laboratory performed antibiotic susceptibility testing as per the CLSI guidelines. No vancomycin resistance was ever reported at this site. All blood culture results were entered into the JEEVA laboratory information system, where results could be accessed by clinicians in the wards and data could be retrieved to assess patterns of antimicrobial resistance. Blood culture data entry system lacked quality control checks hence numerous errors and missing data were observed. Our results support the relevance of having improved laboratory procedures and good quality blood culture since surveillance of antimicrobial resistance primarily depends on good laboratory procedures, good quality and reliable blood culture data. This would essentially minimise imprecise estimates of rates of antimicrobial resistance at this hospital.

KEYWORDS

Antimicrobial Resistance; Microbiology; Surveillance; Laboratory; Tanzania

1. Introduction

Muhimbili National Hospitals' (MNH) existence dates back to 1910 when it was known as Sewahaji. It is a 900-bed specialised National Referral and University Teaching Hospital [1] that provides tertiary health services to inhabitants of Dar es Salaam region, Tanzania, which harbours an estimated population of 2.5 million people [2]. The hospital admits 1000 to 1200 in-patients per day. Blood cultures at this hospital are not routinely conducted because clinical diagnosis of bacteraemia and empirical antibiotic therapy is the main approach to clinical management. Blood culture is only requested in special circumstances, mostly due to treatment non-response or in neonatals due to non-specificity of clinical symptoms in this age group. The aim of this study was to describe in details laboratory methods and procedures relating to blood cultures and their potential impact on antimicrobial resistance surveillance among nosocomial bacteria.

1.1. The Central Pathology Laboratory

The Medical Laboratory Services in Tanzania (called

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Tanganyika during the colonial era) were established in the late 19th Century during the German administration. The first Government Health Laboratory was established in 1897, at Ocean Road in Dar es Salaam. Historically, this laboratory was the first site of a medical laboratory in Tanzania. The laboratory was often visited by Dr Robert Koch who worked in the laboratory on several occasions as he was investigating tropical diseases such as malaria, sleeping sickness etc, which were then a major health problem in the country. Laboratory services have grown and been expanded countrywide. The Ocean Road Laboratory became the Central Pathology Laboratory (CPL) in the early 1960s and is still operational under the Ministry of Health and Social Welfare [3]. The CPL located at the MNH is a key player in the provision of high quality of laboratory services to all patients referred to and admitted at MNH or attended to as out-patients.

1.2. Departments and Laboratory Information System

The CPL is the leading provider of diagnostic laboratory services in Tanzania. In addition, the CPL offers referral laboratory services for tests requests from other public and private hospitals within Dar es Salaam and surrounding regions. The services provided by the CPL are: microbiology, histopathology, parasitology, haematology and blood transfusion, clinical chemistry etc. The CPL uses a laboratory information system (LIS) fully interfaced with all automated diagnostic machines and hospital information management system (HIMS), the Jeeva system 2000 [3]. The system was established as an attempt to improve laboratory services such as turnaround time for laboratory results.

All clinical departments are computerised and interlinked to the LIS and the results are entered and posted on the Jeeva LIS for clinicians to access directly in the wards and other clinical departments through logging into the system with their username and password. The clinicians view the results online in the wards, and this expedites the clinical decision regarding treatment modalities for bacteraemia cases. Hard copies of the laboratory results are sent to the wards afterwards for filing in the patient's files and cross referencing in case of a future episode of an illness. The microbiology unit at the CPL handles high volumes of laboratory results ranging from samples of blood, cerebral spinal fluids, pus swabs, urine specimens, stool etc. The microbiology unit does the following tests among others: bacterial identification, antibiotics susceptibility testing and serological tests. The LIS helps to ensure that results are captured in time and transmitted or released to the patients within acceptable time limits [4].

2. Methodology

2.1. Design and Study Setting

A systematic audit of blood culture procedures and practices was carried out in the department of microbiology of the Central Pathology Laboratory of Muhimbili National Hospital. The audit lasted 3 days and focussed on the procedures and practices carried out in the process of dealing with blood cultures *i.e.* tracing a pathway from receipt of blood culture specimen in the microbiology laboratory to processing the blood culture to communicating results to the clinicians in the wards and entering results on LIS. Our study focussed on blood culture from bacteraemia caused by *Staphylococcus aureus* (SA) and *Klebsiella pneumoniae* (KP).

2.2. Data Collection Procedures

We used a standard guide as we went through different sections of the microbiology department focussing on how blood cultures are done and how data are gathered in the laboratory and utilised for surveillance. The audit involved 1) a comprehensive orientation on the activities of the bacteriology section to familiarise with standard routines and laboratory practice, 2) observation of how blood culture procedures are done in the laboratory and, 3) individual discussions with staff involved in technical procedures of blood culturing and data entry of blood culture results.

3. Results

3.1. Blood Culture Specimen Flow

We schematically describe in the chart below specimen flow of blood cultures and related procedures pertaining to blood cultures at MNH.

The narration of **Figure 1** is provided by the following steps:

Step 1: Two blood culture bottles are collected and sent to the laboratory. For children, only a single specimen is collected into a special blood culture bottle. Duplicate specimens in this laboratory are rare as blood cultures are collected on special request only not as a routine test.

Step 2: From the wards, all specimens are delivered to the laboratory reception area where they are sorted out based on the type of the specimen.

Step 3: At the reception area, blood culture specimens are isolated from the pool of other specimens by the laboratory clerk responsible for all microbiology specimens. A serial number is allocated and pasted onto each of the blood culture specimen bottle.

Step 4: The laboratory clerk then enters patient demographic details from a specimen order form into a register
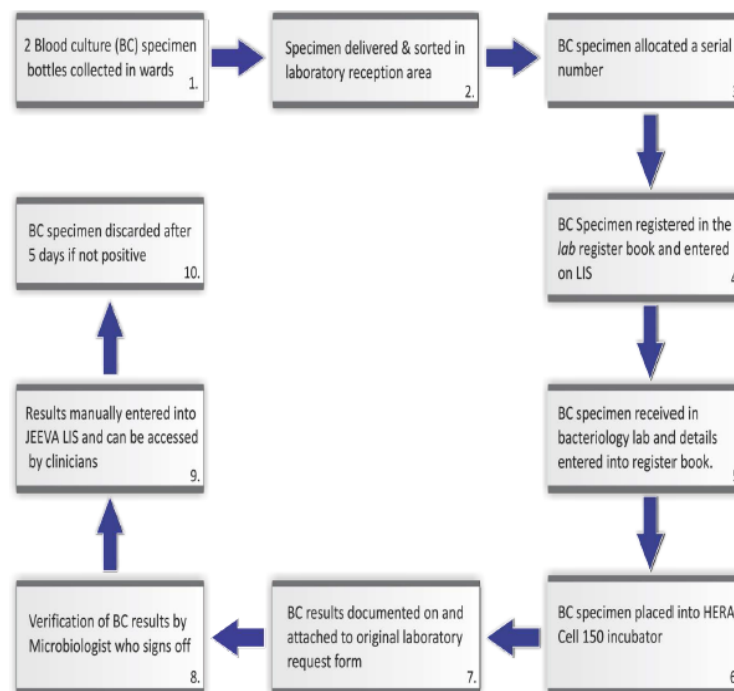
Figure 1. Blood culture data flow and interlinkage with the LIS at MNH microbiology laboratory.

book and LIS database. Once this is done the specimen is then delivered to the bacteriology laboratory for processing.

Step 5: In the bacteriology laboratory, the technician receiving the specimen then enters the patients' details into yet another register book so as to track samples and minimise loss.

Step 6: Blood culture specimen is then placed into the HERA CELL 150 incubator and physically monitored each morning to detect bacterial growth. If visible signs of positive culture are noted, the specimen is taken out for gram staining and susceptibility testing.

Step 7: The results of blood cultures (both positive and negative specimens), are documented on the blood culture results form, which is then attached to the original laboratory request form.

Step 8: Verification of blood culture results is done by the Microbiologist who heads the department or his immediate representative. Once results are signed off, the results are ready to be released to the wards.

Step 9: The blood culture results are handed back to the laboratory clerk who manually enters them into the JEEVA LIS. The electronic record is linked to the ward such that the clinicians in the ward can access the results directly online through the LIS computer network installed in the wards. The hard copies of the results are

also sent to the requesting clinician.

Step 10: The blood culture specimen is discarded after 5 days once no indication of positivity is observed. All necessary protocols for blood cultures are followed so as to minimise error.

3.2. Sample Volumes

Muhimbili National hospital is a large and busy hospital, however the number of blood culture specimens received each day by the microbiology department is in the region of 25 - 30, this gives a clear indication that blood culturing is not a routine practice. In the wards, blood culture is only requested in specific clinical circumstances such as failed empirical antibiotic treatment. As per information from the laboratory register, it was clear that more blood culture requests originate from the paediatrics departments' neonatal unit.

3.3. Blood Culture Processing

Susceptibility testing of isolates is dependent on the availability of disk panels and therefore not all isolates are tested for resistance to all antibiotics. Sometimes testing is only done on second line drugs which are not the standard of care in the hospital. The results of these tests are therefore of little help to clinicians who manage

patients with blood born infections. It was observed that all *Staphylococcus aureus* isolates were tested for vancomycin resistance as a way of monitoring emerging vancomycin resistant *Staphylococcus aureus* (VRSA) [5,6].

3.4. Common Antibiotics Tested

The most common antibiotics subjected to susceptibility testing of blood culture isolates at this hospital were: 1) *Staphylococcus* **species**: amikacin, penicillin, ampicillin, cloxacillin, tetracycline, erythromycin, gentamicin, cephalothin, chloramphenicol, vancomycin; 2) *Klebsiella* **species**: ampicillin, chloramphenicol, tetracycline, amikacin, amoxyclav, cefuroxime and imipenem; 3) *Pseudomonas* **species**: ampicillin, amikacin, ciprofloxacin, chloramphenicol, gentamicin and cotrimoxazole; 4) *Escherichia coli*: chloramphenicol, gentamicin, amikacin, ampicillin and cefuroxime [7].

3.5. Antibiotic Susceptibility Testing

The antibiotic susceptibility testing procedures at this site are done in accordance with the Clinical Laboratory Standards Institute (CLSI), 2010 guidelines [7]. The minimum inhibitory concentration (MIC) for each antibiotic to determine cutoff for antibiotic resistance as outlined in these guidelines are followed. External quality control is done on a regular basis using specimens from the Centres for Disease Control (CDC) to ascertain validity and reliability of antibiotic susceptibility results produced by this laboratory.

3.6. Challenges in Blood Culturing

3.6.1. Automated Laboratory Equipment
Lack of a functioning automated microbial detection system was observed to be important obstacles to effective blood culturing at this laboratory. The automated blood culturing equipment is often not in good working order and servicing takes long time to be done. The laboratory often relies on manual blood culturing technique which has its own limitations such as subjective determination of a positive culture through visual assessment. Accuracy is dependent on individual technicians' visual acuity, hence subject to over or under estimations of true positives. However, it should be noted that manual blood culturing are still the most common mode of blood culturing in most resource constrained countries.

3.6.2. Blood Cultures Results
As per records entered into the blood culture register book, a high rate of negative cultures was observed. This might be due to prior antibiotics use before a blood culture specimen was taken or it might be a true representation of negative blood cultures. In addition, we also noted that there was a high rate of coagulase negative *S.aureus*

which might be due to contamination of the blood culture samples at the point of collection.

3.7. Common Challenges and Errors in Blood Culture Data Recording

The outline here give some of the common challenges and errors in data recording found at MHN, microbiology laboratory.

- There was lack of a standardised way of entering data. For example, age was entered as date of birth, age in months, days, years, etc. It was also often just documented as adult or child. This created confusion in terms of knowing the exact ages of the patients who had blood culture done.
- Missing data was a big issue as information on gender, age, hospital ward; type of organism and clinical data was often not available.
- Lack of standardised reporting of the blood culture results *i.e.* results would be reported differently yet it meant the same: "no bacterial growth; Negative, NBG" etc.
- Different data types were entered into the register and also only a few of the positive blood cultures had sensitivity results entered.
- Lack of specific dates that specimens were taken e.g. the record would just show month *i.e.* July but no specific date mentioned.

3.8. Standard Operating Procedures

The microbiology department operates on principles laid down in the standard operating procedures (SOPs) manual. These procedures are overseen by a Quality Control Officer, who is a member of the team in the microbiology laboratory. The controlling officer is responsible for effecting and approving any changes to the SOPs. Implementation of the SOPs is overseen by the Microbiologist heading the department. The manuals are kept in the microbiology laboratory for ease of reference by all team members.

3.9. Challenges with Data Quality

3.9.1. Laboratory Information System (LIS) Data Entry Format
Jeeva ("Life") Informatics Solutions LLC is a Bioinformatics solutions provider. The software company was founded by Dr Harsha K Rajasimha and is situated in Montgomery County, Maryland, United States of America and is specialised in providing "On-Demand Virtual Bioinformatics Core Facility", genomics bigdata management, analysis, and interpretation [8]. Entry of blood culture results into the JEEVA LIS database was done by a single individual. There was no verification of data entered by a second individual to check for accuracy of

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data entered and to allow for timely correction of errors. The system does not have check codes to control data that is being entered. For example, a characteristic such as "age" the system could take in data in any numerical format such as absolute age, year/date/month, year, months and days. This was certainly problematic and a huge source of error.

3.9.2. Clinical Data

There was often no documentation of patients' prior antibiotics use before a blood culture sample was taken. No provisional diagnosis was captured on the laboratory request form. Should the laboratory request form have some clinical history documented, such information would not be captured onto the system as the database structure of the LIS was not programmed to capture such information.

3.9.3. Determination of Nosocomial Bactereamia

There was no documentation on laboratory request forms on duration of in hospital stay prior to blood culture specimen being taken. Lack of this information makes it difficult to separate nosocomial from community acquired bacteraemia. In so doing the burden of antimicrobial resistance due to nosocomial infection becomes difficult to effectively ascertain.

4. Discussion

Surveillance of antimicrobial resistance monitors changes in microbial populations, allows for the early detection of resistant microbial strains of public health relevance, and supports the prompt notification and active investigation of outbreaks of resistant bacteria [9]. Surveillance of antimicrobial resistance is primarily dependant on good laboratory procedures, good quality and reliable routine blood culture data. To improve the quality of blood culture data and minimise improper estimates of antimicrobial resistance, it is essential that important steps be taken to improve the system of specimen collection at the point of care, registration and blood culture procedures in the microbiology laboratory [10]. High rates of specimen contamination as evidenced by more coagulase negative culture results, this calls for the need to proactively improve blood culture specimen collection procedures as this would ultimately lead to a reduction in blood culture contamination, and provide proper estimates of bacteraemia episodes and rates of antimicrobial resistance.

There is a need to place special emphasis on appropriate completion of blood culture request forms by clinicians in the wards, specimen registration by laboratory clerk and accurate entry of blood culture results by laboratory technicians. Accuracy of blood culture results could also be improved if the automated blood culture machines were functioning properly. Improvements in qual-ity of data could also be enhanced through improved data entry process into LIS either by introducing another software such as WHONET free access software developed since 1989 by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance specifically for antimicrobial susceptibility monitoring plus introducing a system of validating data entered into the LIS [11].

5. Conclusion

The LIS needs check codes so that the system is able to track errors on data entry. Simple improvements in the current system could update the system to be an effective surveillance tool to help monitor development and spread of antimicrobial resistance [12] among blood borne pathogens in Tanzania. Such information in the long run will help in policy formulation around antimicrobial usage to contain the growing crisis of antimicrobial resistance in the country.

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Appendix 12.3: Antimicrobial Resistance Surveillance among Nosocomial Pathogens in South Africa: Systematic Review of Published Literature.

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REVIEW ARTICLE

Antimicrobial Resistance Surveillance among Nosocomial Pathogens in South Africa: Systematic Review of Published Literature

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There has been a significant increase in the prevalence of antimicrobial drug resistance in sub-Saharan Africa. This may increase health-care costs due to patients' needs for more diagnostic tests, longer hospitalization, and poor outcome. Therefore, monitoring systems for resistance patterns are needed to effectively minimize poor outcome. A systematic review was conducted to find out the prevalence of antimicrobial drugs' resistance among *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, and to understand whether or not such data were part of an ongoing surveillance system for nosocomial infections in South Africa. An online search of main databases, including Cochrane Library, PUBMED, and MEDLINE, was done using the following search terms: "antimicrobial resistance" and "surveillance"; "antimicrobial susceptibility" and "surveillance"; *Staphylococcus aureus* or *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*; "nosocomial" or "hospital acquired"; or South Africa or Africa. We also performed manual search of local conferences, theses, and dissertations to identify relevant articles. In total, 41 manuscripts were identified of which eight were analyzed. There is no evidence of any ongoing antimicrobial resistance surveillance for nosocomial pathogens in South Africa. Data reported in this review seem to have been analyzed on an ad hoc basis and do not show a particular resistance pattern; however, data show evidence of resistance to commonly used antimicrobial drugs in this population: for *S aureus*, resistance to cloxacillin was 29% and to erythromycin 38%; for *K pneumoniae*, resistance to ciprofloxacillin was 35% and to ampicillin 99%; and for *P aeruginosa*, the mean resistance to ciprofloxacillin was 43% and to amikacin 35%. Surveillance of antimicrobial resistance is essential to better understand the complexity of antimicrobial resistance development. Such evidence would be used in developing an effective surveillance program to monitor patterns and trends of resistance over time.

1. Introduction

Antimicrobials are essential for the treatment of infectious diseases. However, a high prevalence of resistance impacts patient outcomes negatively. Antimicrobial resistance increases health-care costs due to a need for more diagnostic tests, additional drugs for treatment, and longer duration of hospitalization.[1,2] Therefore, the emergence and spread of antimicrobial-resistant organisms from hospital to the community is a growing public health challenge in South Africa and worldwide. It is associated with a high level of morbidity and mortality, and for this reason, antimicrobial resistance requires effective monitoring to determine patterns and trends over time.[3−6] For South Africa, such information is particularly important because of the HIV/AIDS epidemic and increased antimicrobial consumption due to frequent episodes of opportunistic infections.

Antimicrobial resistance surveillance is crucial for evaluating the use of empirical antimicrobials for treatment.[7] Continuous monitoring, and a better understanding of the profile and magnitude of antimicrobial resistance are therefore required. This will help address the problem of increasing rates of antimicrobial resistance in South Africa. The European Antimicrobial Resistance Surveillance System (EARSS) is an electronic laboratory information system that has been used as a tool for identifying emerging antimicrobial resistance.[8] In South Africa, an equivalent national surveillance system to monitor the status of antimicrobial resistance for nosocomial pathogens has not yet been established. For this reason and as an interim exercise, this review was initiated to gather scientific evidence of the extent and patterns of

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antimicrobial resistance in selected hospital-acquired pathogens in South Africa.

2. Methodology

2.1. Online search strategy

A comprehensive search of biomedical databases was carried out to find all relevant manuscripts published in English. The search aimed at identifying relevant peer-reviewed epidemiological studies that would provide adequate information on antimicrobial surveillance initiatives in South Africa.

2.2. Search engines, dates of publications, and search words used

The following search terms were using: "antimicrobial resistance" and "surveillance"; "antimicrobial susceptibility" and either "surveillance" or *Staphylococcus aureus*" or "*Klebsiella pneumoniae*" or "*Pseudomonas aeruginosa*"; "nosocomial" or "hospital acquired"; or "South Africa" or "Africa." We focused on searching pathogen-specific literature and data for this review using manuscripts identified through such an extensive search of the following databases: Cochrane Library (July 2011); MEDLINE (1966 to July 2011); *African Journals Online* (AJOL) (1980 to July 2011), EMBASE (1980 to July 2011); and LILACS (1982 to July 2011) on www.bireme.br.

2.3. Manual search strategy

We also carried out a manual search and review of the reference lists of the identified articles. Additionally, as findings of studies are not always published conventionally, we manually searched the abstracts and proceedings within the past 10 years for the following conferences: "OIE International Conference on Antimicrobial Resistance," "Conference on Antibiotic Resistance Prevention and Control" (ARPAC), "Public Health Association of Southern Africa" (PHASA), "Federation of Infectious Diseases Society of South Africa" (FIDSSA), "Global Antimicrobial Resistance Program" (GARP), "Congress of the European Society of Clinical Microbiology and Infectious Diseases" (ESCMID), and the "Congress of the International Society for Infectious Diseases." Such conference proceedings outline major group sessions for microbiology and infectious disease specialists working within the field of antimicrobial resistance. We did not obtain any relevant data from these searches. In addition, informal approaches were made to individuals and organizations within the field of hospital infection control and antimicrobial resistance surveillance for information regarding unpublished data, dissertations, and theses.

This search yielded four of the eight papers that were included for analysis. Data for rates of antimicrobial resistances were presented as means.

3. Results

3.1. Antimicrobial resistance surveillance for invasive pathogens in South Africa

A good surveillance system for antimicrobial resistance monitoring should involve ongoing collection and collation of both clinical and microbiological data, with an emphasis on timeliness, accuracy, consistent and standardized methods of collection, and analysis, using a centralized laboratory with appropriate control measures, with a focus on reporting on nosocomial pathogens. Such a system has not been present in South Africa. However, although different methods were used, they were all approved by the National Committee for Clinical Laboratory Standards (NCCLS), predecessor

of the Clinical Laboratory Standards Institute (CLSI), and therefore suitable for trend analysis e.g. ciprofloxacin resistance in *K. pneumoniae* increased in academic hospitals from 18% (24/1324 isolates) in 1999 to 28% (498/1778) in 2007.

From the included studies, lack of clinical data and quality assurance information are deficiencies requiring attention; nonetheless, some steps have been taken to contain resistance development. Prudent use of antimicrobials (antimicrobial stewardship) has been looked at through the South African Society of Clinical Microbiology, formerly the National Antimicrobial Surveillance Forum (NASF), using passively collating antimicrobial data in public through the National Health Laboratory Services (NHLS) and in private health-care sectors through private microbiology laboratories.

The Antibiotic Study Group of South Africa has been active since 1976[9]; this group joined private sector surveillance in 2002 as NASF, meeting and sharing information, and several publications in the area of antimicrobial resistance have been released.[9–12] More recently, the Group for Enteric, Respiratory and Meningeal Diseases Surveillance (GERMS-SA), an established entity within the National Institutes for Communicable Diseases (NICD), has been established, which operates in all nine provinces, focusing on surveillance of community-acquired pathogens and monitoring resistance profiles. As of 2010, a surveillance to monitor resistance among *S aureus* and *K pneumoniae* was established as part of GERMS-SA. Another initiative was introduced in KwaZulu Natal for surveillance of *Escherichia coli* in 2000/2001,[13] and the Veterinary Surveillance of Antimicrobial Resistance in South Africa has been involved in monitoring resistance among zoonotic infections.[14] Table 1 illustrates hospitals and laboratories that contributed antimicrobial susceptibility data for the studies that were included in this review.

3.2. Description of study settings and study designs[12,15–22]

A total of 41 manuscripts were identified: 26 identified through database searches and 14 through manual searches in libraries and among personal contacts. Twenty-four manuscripts were excluded, leaving 18 that had full-text article reviews to further assess for eligibility, and 10 more were further excluded. Eight manuscripts published between 2000 and 2011 were identified and included in this review (Figure 1). Of the eight manuscripts, five were published prior to 2007. All manuscripts identified for this review included

Table 1 Public and private sector laboratories that participated in antimicrobial susceptibility data over the period 2000–2011

Public sector hospitals/ NHLS laboratory*	Private sector laboratories[†]
Chris Hani Baragwanath Hospital	Drs Bouwer & Partners (Ampath)
Charlotte Maxeke Johannesburg Academic Hospital	Drs Dietrich & Voigt (Pathcare)
Steve Biko Academic Hospital	Drs du Buisson, Bruinette & Partners (Ampath)
Dr George Mukhari Hospital	Drs Mauf & Partners (Lancet)
Pelonomi & Universitas Hospital	Drs Swart & Marais (Ampath)
Groote Schuur Hospital	Drs van Rensburg Pathologists
Tygerberg Hospital	Drs Vermaak & Partners
Green Point NHLS Laboratory	Niehaus & Botha
King Edward VIII	
No. 1 Military Hospital	

NHLS = National Health Laboratory Service.
* NHLS from Gauteng province (Johannesburg, Pretoria), Free State province (Bloemfontein), and KwaZulu Natal province (Durban and Western Cape province (Cape Town); † Private laboratories in Gauteng province (Johannesburg, Pretoria), KwaZulu Natal province (Durban), Western Cape province (Cape Town), and Free State province (Bloemfontein).
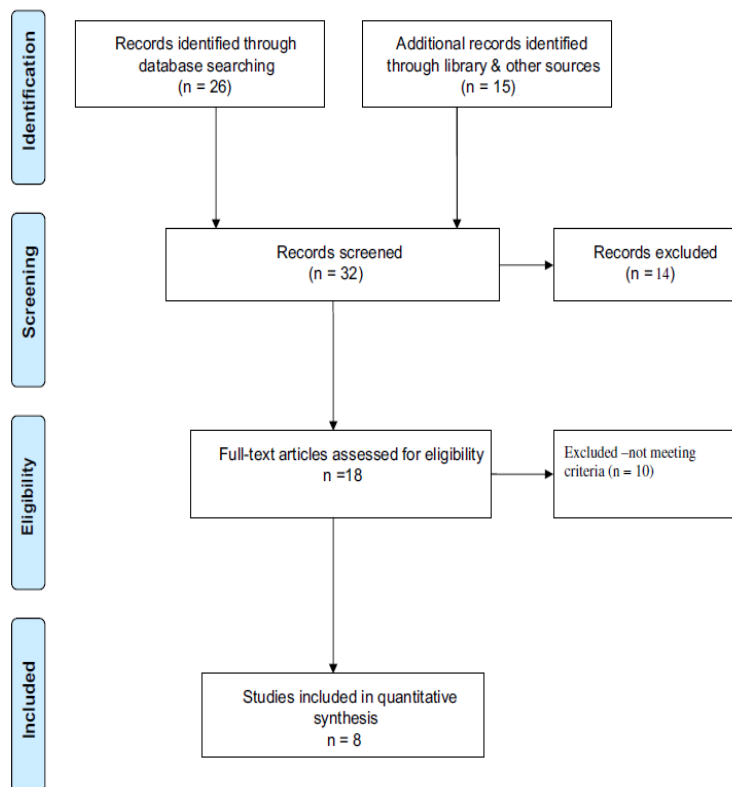
Figure 1 Flow diagram of antimicrobial resistance studies included in the review. *Note.* From PRISMA: www.prisma-statement.org.

susceptibility data from only four of the nine provinces of South Africa. Five of these studies were from public sector tertiary hospitals and three were from private sector laboratories, predominantly from urban settings across South Africa (Table 2).

Seven of these studies produced results from surveillance data aggregated from more than seven sites nationwide, while one study produced results from surveillance data from 16 hospitals within KwaZulu Natal province. None of the eight studies detailed the study design used, other than stating that the study was "multisite and used data of blood culture isolates from microbiology laboratories." Only one study used isolates from respiratory aspirates[20]; all except one study from various public sector hospitals within KwaZulu Natal province used retrospective laboratory data[21] (Table 2).

3.3. Description of microbiological methods[12,16−19,22]

Seven of the studies used data from blood and cerebral spinal fluid (CSF) cultures[12,16−19]; one study used data from respiratory aspirates.[22] The methodologies of antibiotic susceptibility testing

Table 2 Characteristics of antimicrobial resistance studies in South Africa

Author	Year	Pathogen	Location	Sample type	Source of information	Study design
Bamford et al[16]	2009	SA, KP, PA & others	8 NHLS labs	Blood & CSF	NHLS surveillance data	Not specified
National Antimicrobial Surveillance Forum[22]	2008	SA, KP, EC & others	Private labs, no. of labs involved not mentioned	Blood & urine	Private labs data	Not specified
Brink et al[17]	2007	SA, KP,PA & others	7 private laboratories	Blood	Private labs data	Not specified
Sein et al[19]	2005	SA, KP, EC & others	7 NHLS labs	Blood & CSF	NHLS surveillance data	Retrospective approach
Essack et al[21]	2005	SA, KP, PA & others	Laboratories in 16 hospitals	Blood	Public sector surveillance data	Multicenter study in SA
Liebowitz et al[20]	2003	KP & others	12 private labs	Sputum, bronchial brush, BAL, pleural fluid, sinus tap, MEF, pharyngeal swabs	Private labs data	Multicenter study in SA
Crewe-Brown et al[18]	2001	SA, KP, EC & others	8 NHLS labs	Blood & CSF	Public sector surveillance data	Not specified
Antibiotic Study Group of South Africa[12]	2000	SA, KP & others	8 NHLS labs	Blood & CSF	Public sector surveillance data	Not specified

BAL = bronchial alveolar lavage; CSF = cerebral spinal fluid; EC = *E coli*; HI = *Haemophilus influenzae*; KP = *K pneumoniae*; MEF = middle ear fluid; NHLS = National Health Laboratory Service; PA = *P aeruginosa*; SA (pathogen) = *S aureus*; SA = South Africa; SP = *Streptococcal pneumonia*.
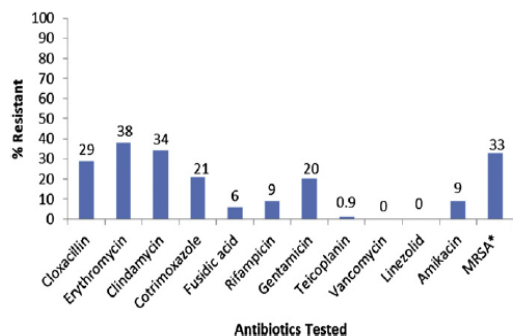
Figure 2 Prevalence of antimicrobial resistance among *S aureus*. *Note*. From seven published studies between 2000 and 2009. *Different methods used to determine MRSA status (Cloxacillin resistance of 29% vs 33% MRSA).

were described in seven studies, all of which mentioned the use of the CLSI breakpoints, formerly NCCLS, to determine antimicrobial susceptibilities. Two studies described in detail other methods used for susceptibility testing of various antibiotics such as Kirby−Bauer disk diffusion, Broth microdilution, E-test, and use of automated Vitek 2 system.[17,20] Only one study mentioned quality control in identification and susceptibility testing as per CLSI recommendations.[17] All studies used only one sample per patient; hence, duplicate samples were excluded to minimize over-representation of the cases that had multiple and frequent cultures. Two studies that reported antimicrobial susceptibility of respiratory tract pathogens mentioned intermediate- and high-level resistance for such organisms.[18,20]

3.4. Resistance rates for different pathogens

3.4.1. Staphylococcus aureus[12,16−19,21,22]
Susceptibility data for *S aureus* were reported in seven studies (Table 2). Five of these studies were from public sector laboratories and two from private sector laboratories.[12,16,18,19,22] Geographically all studies identified were performed in urban areas except one study done in Durban, which included isolates from district and regional hospitals. Specimen types included blood and CSF, except one study that included respiratory aspirates (Table 2). The resistance rate of *S aureus* to cloxacillin was 29%, erythromycin 38%, and

gentamicin 20%, and methicillin resistance (MRSA) was 33%. No resistance has been reported to linezolid since its introduction in 2000, while frequency of resistance to glycopeptides is uncertain due to disagreement on optimization of vancomycin susceptibility testing (Figure 2).

3.4.2. Klebsiella pneumoniae[12,16−22]
Most studies that reported on susceptibility patterns for *K pneumoniae* were published by the Antibiotic Study Group that used data mostly from large public sector academic hospitals that provide services to a diverse population group. Clinical isolates were predominantly from blood and CSF culture (four studies), blood culture only (one study), blood and urine culture (one study), and respiratory aspirates (one study). The resistance of *K pneumoniae* to ciprofloxacillin was 35%, cefuroxime 52%, gentamicin 50%, and ampicillin 99%. Resistance was almost nonexistent for imipenem, meropenem, and moxifloxacin (Figure 3).

3.4.3. Pseudomonas aeruginosa[16,17,21]
Three studies reported resistance rates for *P aeruginosa*, two of which were from blood culture isolates and one from nonspecific sources.[16,17,21] The resistance among *P aeruginosa* to ciprofloxacillin was 43%, gentamicin 50%, amikacin 35%, and aztreonam 42%. Resistance to polymyxin was <5% and was reported in a single study.[16,17,21] Resistance rates to almost all drugs tested were greater than 30% (Figure 4). A study conducted by Perovic et al using data from 1998 to 1999 at Chris Hani – Baragwanath Hospital showed that there was an association between *P aeruginosa* bacteremia and outbreaks caused by multiple-resistant genotypes. In this study, the proportion of nosocomially acquired infection was 57.1%.[24] The resistance profiles and incidence of disease are likely to have changed during the 10-year period, and the current status may be different but is unknown. This review shows high resistance rates of *P aeruginosa* to most conventional antibiotics.

3.5. Presence of extended-spectrum beta-lactamases

Seven studies reported on extended-spectrum beta-lactamases (ESBLs) in *K pneumoniae*. In academic hospitals the rates of ESBLs increased from 33% (436/1324) in 1999 to 49% (869/1778) in 2007. These studies used the double-disk method and reported resistance rates as high as 59% and 62% in private hospitals and public sector hospitals, respectively. A study conducted by Essack Sabiha at
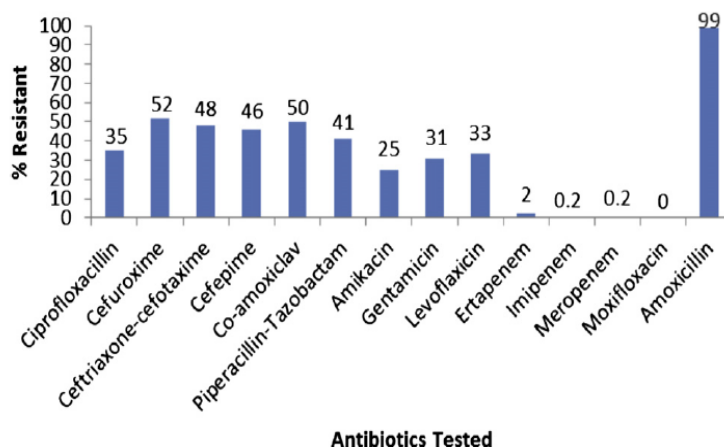

Figure 3 Prevalence of antimicrobial resistance among *K pneumoniae*. *Note*. From eight published studies from 2000 to 2009.
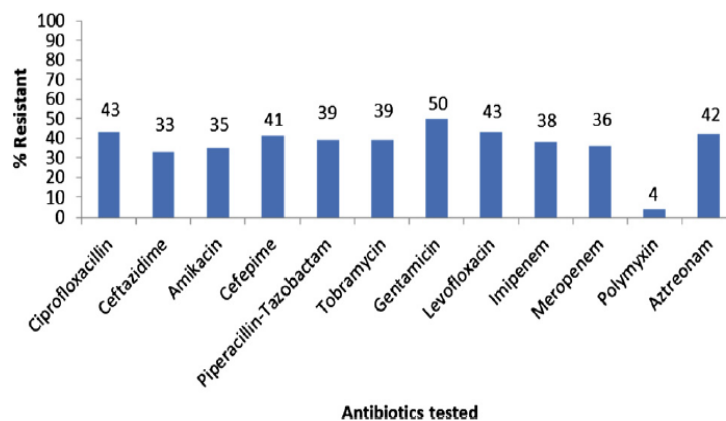
Figure 4 Prevalence of antimicrobial resistance among *P aeruginosa*. *Note.* From three published studies from 2005 to 2009.

a teaching hospital in Durban between 1994 and 1996 investigated ESBL-mediated resistance in South African nosocomial origin of *K pneumoniae* and demonstrated that each of the isolates expressed 1–6 beta-lactamases.[23]

4. Discussion

This systematic review assessed the prevalence of resistance to commonly used antimicrobials as well as whether or not such data were part of an ongoing surveillance system for nosocomial infections in South Africa. We found that no national surveillance system exists that collates and collects data year on year to assess trends and resistance patterns for nosocomial pathogens. In addition, we found that the overall prevalence of resistance to antimicrobials used for empirical treatment is high. Except for polymyxin, with a resistance rate of <5%, most other antibiotics showed high prevalence of *P aeruginosa* resistance to commonly available antimicrobials. The study found a low level of resistance among *K pneumoniae* to moxifloxacin and carbapenems, and a high pattern of resistance to other classes of antimicrobials that are commonly prescribed. *S aureus* showed no resistance to teicoplanin, vancomycin, and linezolid, but high resistance to other classes of antimicrobials. This is similar to the resistance pattern in Central African countries, as shown in a review by Vlieghe et al,[25] even though their study focused mostly on community-acquired pathogens.

Several limitations have been observed in this study: Firstly, studies included in this review reported laboratory data on antimicrobial-resistant isolates, with no clinical data; hence, they could not link resistant isolates to clinical findings. Secondly, most studies aggregated data from different laboratories which employed varied laboratory techniques. This was not ideal for surveillance purposes but all methods were NCCLS/CLSI approved. Thirdly, data used were collected retrospectively, except for a single study by Brink et al that collected data prospectively.[17] Use of retrospective data has several limitations, including incomplete data that are subject to numerous biases. Fourthly, most, if not all, studies lacked demographic data; hence, it was difficult to compare community-acquired versus hospital-acquired infections. Lastly, variation in clinical specimens, taking practices between different institutions, might alter representativeness of data reported from these various studies. Furthermore, this study included invasive pathogens from blood cultures as well as pathogens from respiratory specimens and, in the case of *P. aeruginosa*, also from other sources, including burns."

In spite of the limitations mentioned above, there is growing evidence of escalating rates of antimicrobial resistance to several conventional antimicrobials. Even though vancomycin resistance is still negligible, ESBL and MRSA rates are high in these urban academic centers and private institutions. This emphasizes the fact that surveillance is essential to further our understanding of antimicrobial resistance development and how it relates to prescription practice.[23,25] Such undertaking will pave the way for designing interventions that could overcome resistance development to established antimicrobial agents.

5. Conclusions

Evidence indicates that antimicrobial resistance rate to nosocomial pathogens are generally high in South Africa. This is an emerging threat to public health and clinical management of patients with such infections in the face of dwindling antimicrobial development. We believe that a good surveillance system would enhance effective monitoring of emerging resistance and changes in resistance profiles, and identify significant differences in trends and distribution of antimicrobial resistance.

Authors' contributions

PN searched the relevant papers and drafted the manuscript. JM proposed the topic for this review and helped draft the manuscript. OP and HK participated in critically reviewing the manuscript on intellectual content and scholarly writing. All authors read and approved the final manuscript.

Acknowledgments

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Appendix 12.4: Increased Risk of Death in Human Immunodeficiency Virus-infected Children with Pneumococcal Meningitis in South Africa, 2003-2005.

ORIGINAL STUDIES

Increased Risk of Death in Human Immunodeficiency Virus-infected Children With Pneumococcal Meningitis in South Africa, 2003–2005

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Background: Pneumococcal disease is a major global cause of morbidity and mortality. This study evaluated risk factors for mortality in children with pneumococcal meningitis and other invasive pneumococcal diseases (IPD).
Methods: The study population included patients <15 years of age with laboratory-confirmed IPD and available outcome data between January 1, 2003 and December 31, 2005 as reported to a national laboratory-based surveillance program. Meningitis was defined by having pneumococcus identified from cerebrospinal fluid culture, while other IPD included patients with pneumococci identified from other normally sterile site specimens. Risk factors for mortality were evaluated using multivariable logistic regression.
Results: A total of 2251 patients with IPD were reported from sentinel sites: 581 with laboratory-confirmed meningitis and 1670 with other IPD. The case-fatality ratio was 35% (205/581) among meningitis cases and 18% (300/1670) among other IPD cases ($P < 0.001$). Among individuals with available human immunodeficiency virus (HIV) status data, HIV coinfection was less likely among patients with meningitis compared with other IPD (74% [244/328] vs. 82% [880/1067] $P < 0.001$). On multivariable analysis, HIV-infected status (odds ratio [OR]: 5.34, 95% confidence interval [CI]: 2.32–12.29), Pitt bacteremia score ≥4 (OR: 3.08, 95% CI: 1.21–7.83) and age group <1 year (OR: 2.58, 95% CI: 1.21–5.51) were independent predictors of death among patients with meningitis. Among children with other IPD, malnutrition was an independent predictor of death while HIV infection was not independently associated with increased risk of death.
Conclusions: Pneumococcal meningitis is associated with a high case-fatality ratio among South African children and this is increased by HIV coinfection. Increasing access to antiretroviral therapy and a catch-up program for pneumococcal conjugate vaccine among HIV-infected and malnourished children could reduce this excess mortality.

Key Words: mortality, HIV, meningitis, invasive pneumococcal disease, children, pneumococcus, pediatric

Streptococcus pneumoniae infection is an important cause of death among children and adults worldwide.[1,2] The pathogen is the most common cause of community-acquired bacterial meningitis in children[3] and has been associated with high case-fatality ratios (CFRs) of 45% to 61% in Africa.[4–8] Predisposing factors related to pneumococcal meningitis have included lower respiratory tract infections, sinusitis, mastoiditis, and head injury, whereas malignancies, human immunodeficiency virus (HIV) infection, and asplenia have been identified as underlying conditions.[9–11]

Clinical and laboratory features associated with mortality in children with pneumococcal meningitis identified in previous studies include low cerebrospinal fluid (CSF) white cell count, low CSF glucose value, delay in initiation of treatment, presence of underlying disease, and nonsusceptibility to penicillin.[2,12] For pneumococcal bacteremia, previously identified risk factors for mortality include Pitt bacteremia score ≥4, age, infection due to pediatric serotypes, delay in initiation of treatment, and presence of underlying disease.[13] Published studies from sub-Saharan Africa have shown that the incidence and mortality rates of bacterial meningitis among HIV-infected children are higher than HIV-uninfected children. Madhi et al[5] showed that HIV-infected children less than 2 years old in South Africa had a higher risk of acquiring pneumococcal meningitis compared with HIV-uninfected children. Overall mortality rates among children less than 12 years old with pneumococcal meningitis was significantly higher in HIV-infected compared with HIV-uninfected (30.6% vs. 11.8%). In another study, Molyneux et al[14] showed that HIV-infected children who develop bacterial meningitis had a higher mortality rate compared with HIV-uninfected children (65% vs. 36%, respectively).[14] However, data from the global pneumococcal disease burden study presumed pneumococcal mortality due to IPD to be equal in HIV infected and uninfected.[15] Despite the fact that several studies have looked at clinical outcomes of invasive pneumococcal disease (IPD) in HIV-infected and -uninfected individuals, no study has focused at examining risk factors for death in children in Southern Africa.

The objective of this present study was to investigate independent risk factors for mortality in South African children with pneumococcal meningitis and other IPD.

MATERIALS AND METHODS

Invasive Disease Surveillance

A national laboratory-based surveillance system for IPD was introduced in South Africa in 1999.[16] Laboratories performing clinical microbiology diagnostic tests send reports of laboratory-

confirmed IPD together with isolates to a central laboratory in Johannesburg. Basic demographic details such as age, gender, date of specimen, and source of isolate are collected.

From 2003, the system was enhanced in all 9 provinces. Frequent communications and provincial visits were initiated to increase case reporting. For cases occurring at 15 sentinel hospitals located in 9 provinces, we collected expanded clinical and demographic information including admission date, HIV serologic status, previous antibiotic use, discharge diagnosis, and outcome. In South Africa, the 7-valent pneumococcal conjugate vaccine (PCV7) was licensed in 2005 and was only available in the private health sector until 2008. Use of this vaccine was minimal during the study period. From 2004 onwards, national guidelines recommended the treatment of HIV-infected patients with antiretroviral drugs according to clinical algorithms.[17] Overall prevalence of HIV infection among children <15 years of age in 2005 was 1.9% and did not change substantially during the study period.[18]

This study was a retrospective cohort analysis of surveillance data from January 1, 2003 to December 31, 2005. The analysis used a subset of the data from enhanced surveillance sites with completed case report forms and data on outcome in children less than 15 years of age. Data on antibiotics prescribed for individual case management as well as antibiotics used preceding admission were only collected from January 2005. Data on dosage of antibiotics used were not available.

Case patients were individuals aged <15 years with pneumococcus identified from normally sterile-site specimens at enhanced surveillance sites and with clinical outcome documented. Laboratory-confirmed pneumococcal meningitis was defined as identification of *S. pneumoniae* from CSF, with or without pneumococcus from another normally sterile site. Other IPD was defined as identification of *S. pneumoniae* from any other normally sterile site (eg, blood cultures, synovial fluid, peritoneal fluid, or pleural fluid). Nosocomial infection was defined as specimen collection date ≥ 2 days after date of admission to hospital. Acute severe illness was defined as a Pitt bacteremia score equal to or greater than 4 at the time of specimen collection.[19,20] This score is used to measure severity of disease in bacteremia but was used in meningitis patients as a measure of generalized sepsis. The Pitt bacteremia score was assessed using the following: (i) Oral temperature: 2 points for a temperature of ≤35°C or ≥40°C, 1 point for temperature of 35.1°C-36.0°C or 39.0°C–39.9°C, and 0 point for temperature of 36.1°C to 38.9°C. (ii) Hypotension: 2 points for systolic blood pressure of less than 90 mm Hg. (iii) 2 points for receipt of mechanical ventilation. (iv) 4 points for cardiac arrest. (v) Mental status: if alert 0 point; disoriented 1 point; stuporous 2 points; and comatose 4 points.[19,20]

Malnutrition was defined as the presence of malnutrition as recorded in the medical records. Underlying illness excluding malnutrition and HIV was defined as the presence of malignancy, asplenia, autoimmune condition, organ transplant, or receipt of immunosuppressive agents eg, prednisolone or other cytotoxic drugs, as well as the following illnesses: tuberculosis, diabetes mellitus, chronic renal failure, heart disease, asthma, severe burns, severe trauma, and head injury.[21]

Prior antibiotic use in the past 24 hours was defined as use of any antibiotic within 24 hours before the first positive culture.[19–21] Prior antibiotics use in the past 2 months was defined as use of any antibiotic other than cotrimoxazole prophylaxis within 2 months preceding the first culture-positive specimen. Cotrimoxazole prophylaxis was defined as regular use of cotrimoxazole for purposes of *Pneumocystis jirovecii* pneumonia prophylaxis in HIV-exposed children. Vaccine serotypes were defined as serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F found in PCV7.[22]

Severe HIV immunodeficiency was defined according to World Health Organization criteria as absolute CD4 cell count <1500 cells/mm³ for age ≤11 months; <750 cells/mm³ for age 12 to 35 months, <350 cells/mm³ for age 36 to 59 months, and <200 cells/mm³ for age ≥5 years.[23] Mortality was defined as death within 30 days of admission after the first positive culture result for *S. pneumoniae* was obtained. Patients who were discharged from hospital within or after 30 days were considered to have survived the episode. To minimize misclassification of deaths that may have occurred as a result of causes other than pneumococcal infection, all deaths occurring after 30 days were excluded from the analysis.[24] Patients with missing data on outcome and date of death were excluded from the analysis.

Laboratory Methods

Identification of *S. pneumoniae* was based on standardized methodologies.[25] Isolates were screened for resistance to oxacillin by disk diffusion (Mast Diagnostics Ltd, Bootle, Merseyside, United Kingdom). Penicillin minimum inhibitory concentrations (MICs) were determined for resistant isolates by agar dilutions or Etest (AB-Biodisk, Solna, Sweden). Antibiotic susceptibility for penicillin was defined as "nonsusceptible" referring to intermediate resistance or resistance when MIC were >0.06 μg/mL, and "susceptible" if MIC ≤0.06 μg/mL.[26] Pneumococci were serotyped by the Quellung method using specific antisera (Statens Serum Institute, Copenhagen, Denmark). HIV infection was confirmed according to standard practice at the relevant health care facility. The commonest algorithm included testing using 2 sequential HIV ELISA tests: Determine HIV-1/2 (Abbott GmbH Wiesbaden, Germany) and Murex HIV 1+2 (Murex Diagnostic Limited, Dartford, Kent, United Kingdom).

Statistical Analysis

Analysis was performed using Epi Info, version 6.04d (CDC, Atlanta, GA)[27] and Stata version 10 (StataCorp Limited, College Station, TX). Univariate analysis was performed using Mantel-Haenszel χ^2 tests for categorical variables. Factors that were significant at $P \leq 0.1$ on univariate analysis were evaluated in multivariable logistic regression models. Variables available for evaluation as potential risk factors for death included age group, gender, race, year of infection, province, HIV infection, malnutrition, underlying diseases, Pitt bacteremia score, vaccine serotypes, prior antibiotic use 24 hours before admission, prior antibiotic use 2 months before admission, use of cotrimoxazole prophylaxis, and pneumococcal penicillin nonsusceptibility. Nonsignificant variables at $P < 0.05$ were dropped from the model. The Pearson χ^2 goodness of fit statistic was used to assess model fit. All 2-way interactions in the final multivariable model were evaluated. Two-sided P values of <0.05 were considered significant throughout. As HIV status was the main exposure of interest, we compared patients with HIV status data available to those without available data to explore potential biases introduced through exclusion of patients with missing HIV status data.

For each univariate analysis, we used all available case information. Variables are either binary (yes/no), defined as the presence or absence of the attribute, or have several categories. The study was approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of the Witwatersrand.

RESULTS

Description of Study Population

There were 2251 cases of IPD between 2003 and 2005 among children less than 15 years of age who presented to enhanced surveillance sites and had known outcome. Meningitis was

TABLE 1. Characteristics of Patients With Invasive Pneumococcal Disease (IPD) Presenting to Enhanced Surveillance Sites With Available Data on Outcome, South Africa, 2003–2005

Characteristic	Meningitis n/N (%)*	Other IPD n/N (%)	Odds Ratio (95% CI)
Case-fatality ratio[†]	205/581 (35)	300/1670 (18)	2.48 (2.01–3.07)
Age group (y)			
<1	297/581 (51)	632/1670 (38)	2.40 (1.91–3.01)
1–4	140/581 (24)	714/1670 (43)	Reference
5–14	144/581 (25)	324/1670 (19)	2.27 (1.73–2.96)
Male gender	320/580 (55)	914/1667 (55)	1.01 (0.84–1.23)
Black race	549/1581 (95)	1545/1664 (93)	1.32 (0.88–1.96)
Cotrimoxazole prophylaxis	108/467 (23)	527/1286 (41)	0.43 (0.34–0.55)
HIV infection	244/328 (74)	880/1067 (82)	0.62 (0.46–0.83)
AIDS defining CD4$^+$ counts[‡]	7/47 (15)	44/246 (18)	0.80 (0.34–1.91)
Pitt bacteremia score ≥4	52/412 (13)	64/1141 (6)	2.43 (1.65–3.57)
Pediatric serotype	295/552 (53)	818/1539 (53)	1.01 (0.83–1.22)
Underlying illness[§]	56/581 (10)	435/1670 (26)	0.45 (0.34–0.60)
Malnutrition	20/581 (3)	238/1670 (14)	0.40 (0.33–0.50)
Antibiotic use past 24 h	67/518 (13)	101/1387 (7)	1.89 (1.36–2.62)
Antibiotic use past 2 mo	88/453 (19)	373/1199 (31)	0.53 (0.41–0.69)
Penicillin nonsusceptibility	210/552 (38)	609/1539 (39)	0.94 (0.77–1.14)
Province			
Gauteng	244/581 (42)	712/1670 (43)	Reference
Eastern Cape	61/581 (11)	62/1670 (4)	2.87 (1.96–4.21)
Western Cape	90/ 581 (15)	446/1670 (27)	0.59 (0.45–0.77)
Mpumalanga	38/581 (6)	18/1670 (1)	6.16 (3.45–10.9)
KwaZulu-Natal	73/581 (13)	260/1670 (16)	0.82 (0.61–1.10)
North West	2/581 (0.3)	1/1670 (0	5.84 (0.53–64.6)
Free State	52/581 (9)	156/1670 (9)	0.97 (0.69–1.37)
Limpopo	21/581 (4%)	15/1670 (1)	4.09 (2.07–8.05)
Year of specimen collection			
2003	164/581 (28)	503/1670 (30)	Reference
2004	210/581 (36)	613/1670 (37)	1.05 (0.83–1.33)
2005	207/581 (36)	554/1670 (33)	0.97 (0.83–1.15)

*Number of cases with each characteristics/total number of cases with the syndrome.

[†]Number of deaths/number of cases.

[‡]Severe immunosupression among HIV-positive IPD cases.

[§]Excluding HIV and malnutrition.

TABLE 2. Risk Factors for Mortality in Cases With Pneumococcal Meningitis Presenting to Enhanced Surveillance Sites With Available Data on Outcome, South Africa, 2003–2005

Characteristic	CFR n/N (%)	Univariate Analysis OR (95% CI)	Multivariable Analysis AOR (95% CI)
Age group (y)			
<1	117/297 (39)	1.47 (0.96–2.25)	2.58 (1.21–5.51)
1–4	43/140 (31)	Reference	Reference
5–14	45/144 (31)	1.02 (0.62–1.70)	1.48 (0.63–3.46)
Sex			
Male	118/320 (37)	Reference	
Female	87/260 (33)	0.86 (0.61–1.21)	—
Race			
Black	201/549 (37)	Reference	
Other	5/32 (13)	0.24 (0.86–0.72)	—
HIV status			
Negative	10/84 (12)	Reference	Reference
Positive	93/244 (38)	4.56 (2.24–9.26)	5.34 (2.32–12.29)
Pitt bacteremia score			
<4	112/360 (31)	Reference	Reference
≥4	36/52 (69)	4.98 (2.65–9.35)	3.08 (1.21–7.83)
Vaccine serotype			
No	101/257 (39)	Reference	
Yes	97/295 (33)	0.75 (0.53–1.07)	—
Penicillin susceptibility			
Susceptible	127/342 (37)	Reference	
Nonsusceptible	71/210 (34)	0.86 (0.60–1.24)	—
Underlying illness			
No	186/525 (35)	Reference	
Yes	19/56 (34)	0.94 (0.52–1.67)	—
Malnutrition			
No	196/561 (35)	Reference	
Yes	9/20 (45)	1.52 (0.62–3.74)	—
Province			
Gauteng	95/244 (39)	Reference	
Eastern Cape	27/61 (44)	1.24 (0.70–2.19)	
Western Cape	16/90 (18)	0.34 (0.18–0.62)	
Mpumalanga	17/38 (45)	1.27 (0.64–2.53)	
KwaZulu-Natal	19/73 (26)	0.55 (0.31–0.99)	
Free State	22/52 (42)	1.15 (0.63–2.11)	
Limpopo	7/21 (33)	0.78 (0.31–2.01)	

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

identified in 26% (581/2251) of cases, whereas 74% (1670/2251) were classified as other IPD. Of the patients with other IPD, 97% (1618/1670) had bacteremic pneumonia and 3% (52/1670) presented with pneumococci from other sterile sites (joint or peritoneal fluid) or bacteremia with no identified source of infection. Data on HIV status were available for 62% (1395/2251) of all cases: 56% (328/581) of cases with meningitis compared with 64% (1067/1670) of other IPD cases had data on HIV status (P = 0.001).

The overall CFR was 22% (505/2251). On univariate analysis, children with meningitis had a higher CFR of 35% (205/581) compared with other invasive disease, 18% (300/1670) (odds ratio [OR]: 2.48, 95% confidence interval [CI]: 2.01–3.07) (Table 1). Twenty-five percent (144/581) of deaths among meningitis cases and 11% (186/1670) deaths among other IPD cases occurred within 2 days of the first positive culture. Patients with meningitis were also more likely to be aged <1 year and have an elevated Pitt bacteremia score ≥4, although they were less likely to be using cotrimoxazole prophylaxis, be HIV-infected, have another underlying illness, malnutrition, or prior antibiotic use (last 24 hours). There was marked variation in the proportion of patients with meningitis between the different provinces of South Africa (Table 1).

Comparison of Patients With Data on HIV Status Versus Patients Without Data on HIV Status

The characteristics of meningitis patients with available data on HIV status were similar to those without data on HIV status, except that patients not tested for HIV had a higher CFR, higher Pit bacteremia scores, were less likely to be on cotrimoxazole prophylaxis, and less likely to be infected with penicillin nonsusceptible isolates. In addition, HIV testing varied between the provinces of South Africa and was more likely in more recent years (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A906).

For other IPD, characteristics of patients with available data on HIV status differed significantly from patients without data on HIV status with respect to age group, province of origin, penicillin nonsusceptibility, and year of specimen collection (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A906).

Univariate and Multivariable Analysis of Risk Factors for Death Among Patients With Meningitis

Factors significantly associated with mortality on univariate analysis in patients with meningitis were age group <1 year, black race, Pitt bacteremia score ≥4, Western Cape province, and HIV

TABLE 3. Association Between Baseline Characteristics and Mortality in Cases With Nonmeningitis IPD Presenting to Enhanced Surveillance Sites With Available Data on Outcome, South Africa, 2003–2005

Characteristic	CFR n/N (%)	Univariate Analysis OR (95% CI)	Multivariable Analysis Adjusted OR (95% CI)
Age group (y)			
<1	160/632 (25)	1.90 (1.45–2.50)	1.89 (1.32–2.69)
1–4	108/714 (15)	Reference	Reference
5–14	32/324 (10)	0.61 (0.40–0.93)	0.64 (0.36–1.13)
Sex			
Males	157/914 (15)	Reference	
Females	143/753 (19)	1.13 (0.88–1.45)	—
Race			
Black	285/1545 (18)	Reference	
Other	15/119 (13)	0.64 (0.37–1.11)	—
HIV status			
Negative	30/187 (16)	Reference	
Positive	154/880 (18)	1.11 (0.72–1.70)	—
Pitt bacteremia score			
<4	158/1077 (15)	Reference	Reference
≥4	36/64 (56)	7.47 (4.44–12.60)	7.88 (4.52–13.74)
Vaccine serotype			
No	117/721 (16)	Reference	
Yes	164/818 (20)	1.29 (0.99–1.68)	—
Penicillin susceptibility			
Susceptible	162/930 (17)	Reference	
Nonsusceptible	119/609 (20)	1.15 (0.88–1.49)	—
Cotrimoxazole prophylaxis			
No	128/759 (17)	Reference	
Yes	71/527 (13)	0.77 (0.56–1.05)	—
Underlying illness			
No	255/1235 (21)	Reference	
Yes	45/435 (10)	0.44 (0.31–0.62)	—
Malnutrition			
No	233/1432 (16)	Reference	Reference
Yes	67/238 (28)	2.02 (1.47–2.76)	2.74 (1.83–4.09)
Province			
Gauteng	144/712 (20)	Reference	Reference
Eastern Cape	16/62 (26)	1.37 (0.75–2.49)	1.34 (0.59–3.02)
Western Cape	55/446 (12)	0.55 (0.39–0.77)	0.52 (0.34–0.81)
Mpumalanga	5/18 (28)	1.51 (0.53–4.32)	2.22 (0.53–9.25)
KwaZulu-Natal	50/260 (19)	0.93 (0.65–1.34)	1.16 (0.72–1.87)
Free State	26/156 (17)	0.78 (0.49–1.24)	0.76 (0.41–1.38)
Limpopo	4/15 (27)	1.43 (0.45–4.57)	1.42 (0.36–5.60)

infection (Table 2). On multivariable analysis, HIV-infected individuals with meningitis were 5.34 times more likely to die than HIV-uninfected individuals (OR: 5.34, 95% CI: 2.32–12.29). Age group <1 year (OR: 2.58, 95% CI:1.21–5.51) and acute severe illness measured by Pitt bacteremia score ≥4 (OR: 3.08, 95% CI: 1.21–7.83) were also independently associated with increased odds of death (Table 2).

Univariate and Multivariable Analysis of Risk Factors for Death Among Patients With Other IPD

On univariate analysis of risk factors among patients with other IPD, factors significantly associated with mortality were age group <1 year, Pitt bacteremia score ≥4, underlying illness, malnutrition, and province (Table 3). On multivariable analysis, children of age group <1 year were more likely to die compared with children of age group 1 to 4 years (OR: 1.89; 95% CI: 1.32–2.69). Children with Pitt bacteremia score ≥4 had increased

odds of dying (OR: 7.88, 95% CI: 4.52–13.74). Children with malnutrition were 2.74 times more likely to die compared with those without malnutrition (OR: 2.74, 95% CI: 1.83–4.09) and patients from the Western Cape Province had significantly reduced odds of dying (OR: 0.52, 95% CI: 0.34–0.81) compared with children resident in Gauteng. HIV infection was not independently associated with death among other IPD cases (Table 3).

Antibiotic Treatment of Patients

Data on antibiotics used for case management in 2005 were available for 33% (194/581) of the meningitis cases and 32% (534/1670) of patients with other IPD. Among meningitis cases, 154 (79%) received a third-generation cephalosporin, 38 (20%) received penicillin, and 2 (1%) received a first- or second-generation cephalosporin. The odds of mortality were higher among cases who received penicillin compared with those who received ceftriaxone (OR: 1.18, 95% CI: 0.60–2.30); however, this was not statistically significant. Among other IPD cases, 298 (57%) received penicillin; 148 (28%) received a third-generation cephalosporin; 44 (8%) received a first- or second-generation cephalosporin; and 37 (7%) received other antibiotics including augmentin, ciprofloxacin, cotrimoxazole, metronidazole, gentamicin, or erythromycin. Among other IPD patients receiving penicillin therapy, those who had disease due to penicillin nonsusceptible isolates were more likely to die than those with disease due to penicillin susceptible isolates (OR: 1.19, 95% CI: 0.91–1.55). There was a significant increase in the proportion of penicillin nonsusceptible pneumococci in South Africa over the study period from 32% in 2003, 40% in 2004, and 45% in 2005 ($P < 0.001$).

DISCUSSION

This study evaluated risk factors for death in children with pneumococcal meningitis and other IPD. We found that children with pneumococcal disease in South Africa experienced a high CFR, especially those with meningitis, where more than one-third of identified patients died. In addition, we determined that HIV-infection was an independent risk factor for death in children with pneumococcal meningitis, with HIV-infected children having nearly 5 times greater odds of dying compared with HIV-uninfected children. Although HIV infection was not associated with increased odds of death in patients with other IPD, malnutrition was.

Our results confirm the high burden of mortality associated with IPD. Mortality rates of 21% to 30% in children with pneumococcal meningitis from North America and Europe[3,9,28] have been reported previously and mortality rates of 10% to 47% in children with IPD have been documented in sub-Saharan Africa and the Arabian Peninsula.[29–32] In South Africa, previous reports have documented mortality rates of 36% among children with advanced HIV disease and IPD and 61% among HIV-infected children with pneumococcal meningitis admitted to tertiary care hospitals.[5,33] Mortality observed in our study was greater than that observed in Europe but less than that in previous South African studies. The lower mortality in the present study could be due to introduction of antiretroviral treatment which has led to a decline in the incidence of IPD as well as reduction in associated morbidity and mortality among the HIV-1 infected individuals due to improved immune function.[34,35]

A previous study that evaluated risk factors associated with mortality did not find meningitis as a significant risk factor for death on multivariable analysis among adults.[21] In separate studies evaluating risk factors associated with mortality in patients with IPD, Kalin et al[36] and Moroney et al[37] systematically excluded meningitis from the analyses and mostly looked at the association between bacteremic pneumococcal pneumonia and mortality. The

presence of acute severe illness clinically assessed using the Pitt bacteremia score was associated with mortality for both meningitis and other IPD and might be an indicator of overwhelming systemic disease with inevitable poor outcome.[21,24,37–39]

Age <1 year was associated with greater odds of mortality compared with older children, and this has been reported previously.[12,40] We also found that children residing in the Western Cape Province had lower odds of dying (significant also for other IPD) compared with those residing in Gauteng. Possible differences in health care services including access to care, specimen-taking practices, and case management in these provinces may lead to differential outcomes in patients with meningitis and other IPD.

Penicillin nonsusceptibility was not associated with increased risks of mortality, either in cases with meningitis or other IPD. This finding is consistent with the published literature of previous studies that found no association between increased mortality and other IPD caused by penicillin nonsusceptible pneumococcal isolates.[24,28,37,41] In South Africa in 2005, penicillin was still the recommended therapy for community-acquired pneumonia in children.[42] Third-generation cephalosporins are recommended for empiric therapy for acute bacterial meningitis.[43] Treatment guidelines have not changed during the study period, and no major changes in clinical practice are thought to have occurred.

Several factors might have introduced bias in our study. First, despite collecting data using objective data sources, eg, death certificates and medical records, data on antibiotic use before admission to hospital were collected through person-to-person interviews potentially giving rise to systematic differences in information collected. Second, our sample population, which was patients from enhanced sites with completed case report forms and data on outcome, was different from the patients from enhanced sites without case report forms with respect to race and geographical distribution (data not shown), and this may have introduced bias.

Our study had certain limitations, not uncommon with surveillance data. First, detailed data on severity of illness and other clinical indicators of disease severity, ie, mechanical ventilation, Glasgow coma score, and other parameters such as white blood count were not available. As such, we could not make an in-depth analysis of clinical risk factors associated with mortality. Second, we did not have data on mortality post admission; as such, death due to IPD might have been underestimated. Data on antibiotic dosage and duration of treatment were also lacking making it difficult to establish discordant therapy; hence, we could not fully examine the association between antibiotic resistance and mortality. Incompleteness of information is an inherent weakness of surveillance systems. Data on number of children hospitalized during the study period were lacking; hence, we may have under- or overestimated the burden on IPD due to lack of appropriate denominators, and we were unable to estimate the number of cases without specimens taken or those with culture-negative specimens.

In conclusion, our study has shown that risk factors associated with mortality in children with meningitis include age <1 year, Pitt bacteremic score ≥4, and HIV infection. In patients with other IPD, malnutrition was independently associated with increased mortality. This study provides new information for the identification of populations at risk for death and reinforcement of targeted preventive health services to children, particularly infants. Increasing access to antiretroviral therapy for HIV-positive patients, enhanced measures for the prevention of mother-to-child transmission of HIV infection, and a catch-up program for pneumococcal conjugate vaccine for immunization in children in high HIV prevalence settings[22,44] would help to reduce the incidence as well as excess mortality in at risk groups.

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Appendix 12.5: Conference Presentations

Appendix 12.5.1 Presentation at 14th Congress of the International Federation of Infection Control, Portomaso, Malta

Congress of The International Federation Of Infection Control

in-hospital transfers, and very severe clinical pictures. We suggest that hospital screening for CRE to be extended also to contacts of colonized patients and inappropriate hospital admissions of elders avoided whenever possible.

P39 Prevalence and risk factors associated with antibiotic resistance among nosocomial isolates of Pseudomonas aeruginosa in tertiary public hospitals in South Africa, 2005-2009

Peter Nyasulu[1], Olga Perovic[2,3], Jill Murray[3], Samuel Manda[5], Hendricks Koornhof[2,3]

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[5]Department of Biostatistics, Medical Research Council, Pretoria, South Africa

Aim
To assess prevalence and factors associated with antibiotic resistance among nosocomial isolates of Pseudomonas aeruginosa bacteraemia among patients admitted in tertiary public hospitals in South Africa.

Methods
A retrospective analysis of blood culture isolates of Pseudomonas aeruginosa bacteria from seven tertiary public hospitals was carried out. Data were collated from diagnostic microbiology laboratories of the National Health Laboratory Services between July 1, 2005 and December 31, 2009. Multivariable logistic regression models were constructed to assess significant risk factors associated with antibiotic resistance.

Results
A total of 1561 nosocomial isolates of Pseudomonas aeruginosa were identified over this period. The prevalence of antibiotics resistance was 52% and highest in the 30-39 year age-group and similar between males and females. Sixty seven percent of Pseudomonas aeruginosa resistant isolates were from Steve Biko Pretoria Academic hospital. Mean colistin resistance rate of 1.9% (range 0.0-13.3%) was observed. Pseudomonas aeruginosa resistance to meropenem showed a significant increasing trend from 2006 (27.5%) to 2009 (53.9%) (p<0.001). Age-group less than 5 years, female gender, hospital location and year of infection were significant risk factors associated with higher rates of antibiotic resistance.

Conclusions
The prevalence of antimicrobial resistance was high and showed a significant increasing trend among individual agents i.e. colistin, meropenem, cefepime among others. Continued surveillance of antimicrobial resistance among bloodstream nosocomial acquired infections need to be enhanced. Such data would increase our understanding of the magnitude of the antibiotic resistance and provide objective evidence to re-enforce policies and practices aimed at containing antibiotic resistance.

Key words
antimicrobial resistance, blood culture, nosocomial infection, surveillance, South Africa.

P40 Understanding laboratory methods and their impact on antimicrobial resistance surveillance, at Muhimbili National Hospital, Dar es Salaam, Tanzania

Peter Nyasulu[1,2], Mabula Kasubi[3], Respicious Boniface[4], Jill Murray[2]

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[2]School of Public Health, University of the Witwatersrand, Johannesburg, South Africa
[3]Department of Microbiology, Central Pathology Laboratory, Muhimbili National Hospital, Dar es Salaam, Tanzania

[4]*Muhimbili Orthopaedic Institute, Muhimbili National Hospital, Dar es Salaam, Tanzania*

Introduction
The study sought to describe laboratory methods and blood culture procedures and their impact on antimicrobial resistance surveillance among nosocomial bacteria.

Methodology
We conducted a systematic audit of blood cultures procedures and practices in the Department of Microbiology, Central Pathology Laboratory at Muhimbili National Hospital, between 19th and 23rd March 2012.

Results
The study identified that a total of 25-30 blood culture specimens were received each day an indication of low volumes of blood culturing at this site. More blood culture requests came from the neonatal unit of the hospital, and were performed manually with high culture negative specimens. The laboratory performed antibiotic susceptibility testing as per the CLSI guidelines. No vancomycin resistance was ever reported at this site. All blood culture results were entered into the JEEVA laboratory information system, where results could be accessed by clinicians in the wards and data could be retrieved to assess patterns of antimicrobial resistance. Blood culture data entry system lacked quality control checks hence numerous errors and missing data were observed.

Conclusions
Our results support the relevance of having improved laboratory procedures and good quality blood culture since surveillance of antimicrobial resistance primarily depends on good laboratory procedures, good quality and reliable blood culture data. This would essentially minimise imprecise estimates of rates of antimicrobial resistance at this hospital.

Keywords: antimicrobial resistance, microbiology, surveillance, laboratory, Tanzania.

Clostridium difficile: results of an active surveillance system in Azienda Sanitaria di Firenze

Anna Poli, Enrica Fornai, Elisabetta Paoletti, Denise Borgioli

Health Agency Florence (Azienda Sanitaria Firenze), Italy

Clostridium difficile is one of the major organism of healthcare-associated diarrhea, burden by increased incidence and severity.

Azienda Sanitaria Firenze encourages an active surveillance for CDAD (C. Difficile Associated Disease), since 2010, to measure the phenomenon, identify main risk factors, follow the epidemiological trend in the complications' aspects, re-infections, epidemics and the use of combined and / or prolonged antibiotic therapy.

In 2012 were notified 246 cases of CDAD: 229 patients were aged ≥ 65 years (93.9%), 15 patients were adults <65 years (6,1%). 174 came from their home (70,7%) and 72 from other care settings (29,3%). The risk factors analysis show that those most involved in the development of CDAD is re-hospiatlization in the three months before (152 patients, 61,8%), antibiotic therapy (145 patients, 58,9%), and age ≥ 65 years (9,8%). Acutally our challenge is the management of re-infections: in 2012 were reported 34 cases (13,8%).

Azienda Sanitaria Firenze provides, for the cases the adoption of a strategy of treatment with metrnidazole and vancomycin. 37,8% of cases were treated with metronidazole, 28% vancomycin and 28% with both.

The system of an active surveillance through the feedback of the results becomes an effective tool for analysis and review of the quality of healthcare. Understanding and measuring the phenomenon and identify different areas for improvement encouraging

Appendix 12.5.2 Presentation at 4th ICAN, Cape Town, South Africa

Antimicrobial resistance among clinical isolates of invasive Staphylococcus aureus from seven academic hospitals in South Africa over a 12 months period.

Category: Default
Presentation: Oral
Presenting Author:

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Peter S. Nyasulu	School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg	ZA	+27117172607	peter.nyasulu@wits.ac.za

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Jill Murray	School of Public Health, Faculty of Health Sciences, University of the Witwatersrand & National Institutes of Occupational Health of the National Health Laboratory Service, Johannesburg	South Africa	+27117126400	jill.murray@nioh.nhls.ac.za
Samuel Manda	Department of Biostatistics, Medical Research Council, Pretoria	South Africa	+27716242364	samuel.manda@mrc.ac.za
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Aim: To describe the frequency of antimicrobial resistance patterns of blood culture isolates among Staphylococcus aureus (SA), a hospital-associated bacterial pathogen using microbiology data collected prospectively through an active national antimicrobial resistance surveillance system. Methods: Blood-culture isolates of SA were identified by automated MicroScan, Vitek 2 systems or standard biochemical tests. Antimicrobial susceptibility testing was done following the Clinical Laboratory Standards Institute (CLSI) guidelines. All clinically relevant antimicrobials were systematically investigated for resistance. Results: There were 885 SA isolates reported between July 2010 and June 2011, 60.5% were from Gauteng province. SA resistance to beta lactams was >40% and methicillin resistance (MRSA) was 54.9% but higher (64.5%) in the <5 years age-group, and significantly different across all hospitals ranging (42.9%-85.1%, p=<0.001). Highest rates of MRSA (91/107, 85.1%) were observed at Tygerberg hospital. Resistance amongst daptomycin, fusidic acid, linezolid, synercid, teicoplanin and vancomycin was in general <2% showing development of resistance to antibiotics that SA isolates were otherwise fully susceptible. Conclusions: This study provides objective evidence on the profiles of antimicrobial resistance among SA isolates. Continued surveillance of antimicrobial resistance should therefore be reinforced as such data may be a useful guide for physicians initiating empiric therapy and will guide formulation of antimicrobial prescription policies.

Appendix 12.5.3: 7th PHASA, Sandton, Johannesburg

P 102 - SURVEILLANCE OF ANTIMICROBIAL RESISTANCE AMONG NOSOCOMIAL PATHOGENS IN SOUTH: A REVIEW OF LITERATURE

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Antimicrobial agents remain the key to treatment of infectious diseases. In the era of HIV infection, frequent episodes of bacterial infections has propagated an increase in the prevalence of resistance as a result of increased antimicrobial consumption in the population. Antimicrobial resistance has shown to have a negative impact on patient outcomes, may also lead to an increase in health care costs due to need for more diagnostic tests and longer hospitalization as a result of treatment non-response. To understand the complexity of antimicrobial resistance to nosocomial infections in South African hospitals, we carried out a systematic review to assess existing data published or unpublished on antimicrobial resistance surveillance in South Africa. We focused on unveiling antimicrobial resistance monitoring system for resistance patterns for common nosocomial-associated pathogens these being *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Our review suggests that their is lack of a continuing monitoring system for resistance patterns over time and also that emergence of multiple drug resistance in nosocomial acquired pathogens seems to infer likely links to the spread of resistance within health care settings and to the community. Such findings seem to suggest that it would be ideal to establish an effective monitoring system to determine patterns and trends of resistance over time. Data on antimicrobial resistance is essential to better understand the profile and magnitude of antimicrobial resistance in the population and use the evidence to strategically plan targeted interventions.

Appendix 12.5.4: 15th International Congress of Infectious Diseases, Bangkok, Thailand.

Trends and pattern of antimicrobial resistance among blood culture isolates of selected bacterial pathogens in South Africa, 2005-2009

P. Nyasulu[1]*, O. Perovic[2], J.Murray[3], S. Luchters[1], C. Chasela[1], H.Koornhof [2]

1 University of the Witwatersrand, Johannesburg, South Africa
2 National Institute for Communicable Diseases, Johannesburg, South
Africa
3 National Institutes of Occupational Health, Johannesburg, South
Africa

Background: To investigate prevalence, patterns and time trends of resistance to commonly used antibiotics and factors associated with antimicrobial resistance of selected isolates from blood-specimens collected from patients with bacteraemia and submitted to diagnostic microbiology laboratories at 7 tertiary public hospitals in South Africa.

Methods: We conducted a retrospective cohort analysis of routine data of blood culture-positive *Staphylococcus aureus* (SA), *Klebsiella pneuomoniae* (KP), and *Psuedomonas aeruginosa* (PA) submitted to the National Health Laboratory Services (NHLS) between January 1, 2005 and December 31, 2009. Antimicrobial resistance to commonly used antimicrobials was systematically investigated. Multivariable logistic regressions models were used to assess factors associated with antimicrobial resistance.

Results: A total of 9,969 isolates were reported 3942 (39.5%) SA, 4466 (44.8%) KP and 1561 (15.7%) PA. There were more resistant isolates in 30-39 years age-group for SA 28.4% and PA 51.5%. For KP, 73.3% were in the 5-9 years age-group. SA and PA resistance was similar between males and females, for KP 66.8% were among females; 47.9% SA, 72% KP and 67.1% PA respectively were found to be resistant in three different hospitals from three provinces. SA resistance to ampicillin was >98% and to vancomycin <0.1%. KP resistance to carbapenems was very low: ertapenem 2% (range 0.5%-4.6%), imipenem 0.1% (range 0%-0.5%) and meropnem 0.1% (range 0%-0.3%); and to colistin 1.7% (range 0-2.6%). PA resistance to colistin was 1.9% (range 0 - 13.3%). There was a significant increase in trend of KP resistance to ciprofloxacin (32.6% to 64.9%, p<0.001), cotrimoxazole (67.5% to 81.6%, p<0.001) and cefazolin (80.9% to 95.7%, p<0.00)1. PA resistance to meropenem showed a significant increasing trend from 2006 (27.5%) to 2009 (53.9%) p<0.001. Age group <5 years; female sex; hospital location and year of infection were significantly associated with antimicrobial resistance.

Conclusion: The prevalence of antimicrobial resistance was high among children <5 years old and females with bacteraemia. Enhancement of continued surveillance of hospital acquired infections is therefore recommended as trend of antimicrobial resistance is increasing. Such data would provide understanding of the extent of the problem and present evidence for future policies and practices aimed at containing antimicrobial resistance.

Appendix 12.5.5: 1st Global Forum for Bacterial Infections, India.

CHANGING THE LANDSCAPE OF ANTIMICROBIAL RESISTANCE AMONG NOSOCOMIAL PATHOGENS IN SOUTH AFRICA: SYSTEMATIC REVIEW OF PUBLISHED LITERATURE

Nyasulu P[1], Murray J[1,2], Perovic O[3], Koornhof H[3]

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[2] National Institute for Occupational Health, National Health Laboratory Service (NHLS), Johannesburg, South Africa
[3] School of Pathology, Faculty of Health Sciences, University of the Witwatersrand and National Institute for Communicable Diseases, NHLS, Johannesburg, South Africa.

Introduction
There has been a significant increase in the prevalence of antimicrobial drug resistance in sub-Saharan Africa. This may increase health care costs due to patients' needs for more diagnostic tests, longer hospitalization and poor outcomes. Therefore monitoring systems for resistance patterns are needed to effectively minimize poor outcomes.

Objective
The study was conducted to determine the resistance rate of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to antimicrobial drugs and to understand if these data were derived from an ongoing surveillance system for nosocomial infections in South Africa.

Methods
Online search of databases: Cochrane Library, PUBMED and MEDLINE.
Search terms 'antimicrobial resistance' AND 'surveillance'; 'antimicrobial susceptibility' AND 'surveillance' or '*Staphylococcus aureus*' or '*Klebsiella pneumoniae*' or '*Pseudomonas aeruginosa*'; 'nosocomial' or 'hospital acquired' AND 'South Africa' or 'Africa'.

Fig.1 Inclusion cascade
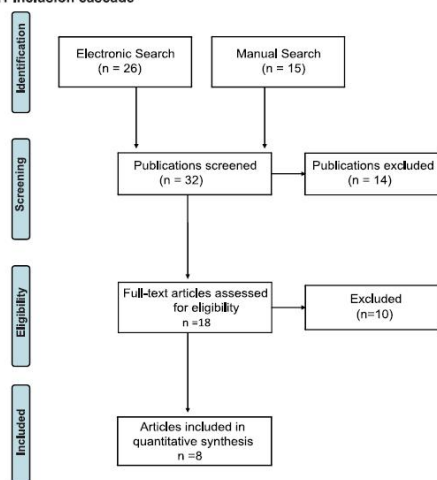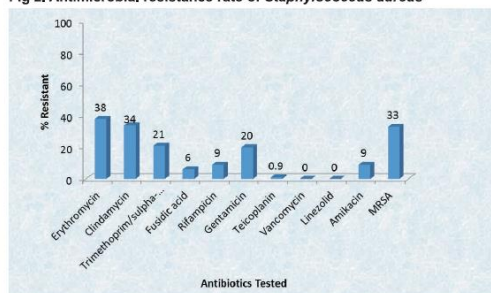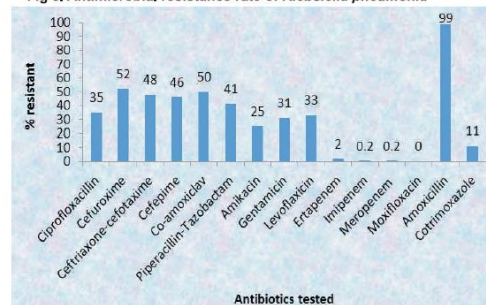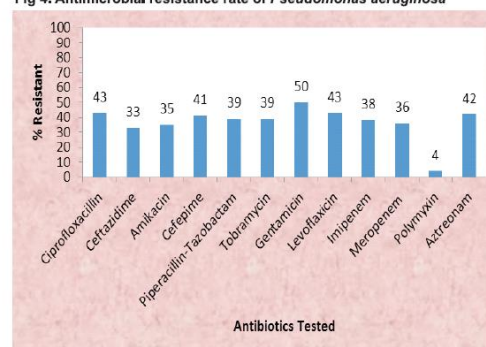
Results
- No antimicrobial resistance surveillance to nosocomial pathogens was identified.
- *Staphylococcus aureus* resistance rate to erythromycin 38%, gentamicin 20%, clindamycin 34% & MRSA 33%. No resistance to teicoplanin, linezolid and vancomycin. (fig 2)
- *Klebsiella pneumonia* resistance rate to ciprofloxacillin was 35%; cefuroxime was 52%, gentamicin 50% and ampicillin 99%. There was almost no resistance to imipenem, meropenem and moxifloxacin. (fig 3)
- *Pseudomonas aeruginosa* resistance rate to almost all drugs tested was >30%. (fig 4)

Conclusion
- There is no antimicrobial resistance surveillance for nosocomial pathogens published up to now.
- These data suggest evidence of high antimicrobial resistance to nosocomial pathogens.

Policy Relevance
- There is an urgent need to establish a surveillance system to monitor patterns and trends of antimicrobial resistance to nosocomial pathogens.

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Appendix 12.5.6: Wits SoPH Biennal Research Day, Johannesburg.

<div style="border:1px solid black">

COMMUNICABLE & NON – COMMUNICABLE DISEASES

</div>

POTENTIAL PREDICTORS OF DEATH IN CHILDREN WITH INVASIVE PNEUMOCOCCAL DISEASE IN SOUTH AFRICA, 2003-2005
<u>Nyasulu P</u>[1], Cohen C[1,2], von Gottberg A[1,2], de Gouveia L[2], Feldman C[3], Klugman KP[2,4] for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa.

[1]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2]National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS), Johannesburg, South Africa
[3]Division of Pulmonology, Department of Medicine, Johannesburg Hospital and University of the Witwatersrand, Johannesburg, South Africa
[4]Hubert Department of Global Health, Rollins School of Public Health, and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, GA, USA

O- 07

Background: *Streptococcus pneumoniae* causes high morbidity and mortality in children, the elderly as well as HIV-infected individuals. The aim of this study was to investigate predictors of death in children with invasive pneumococcal disease (IPD).

Methods: The study population was children aged 0-14 years with laboratory-confirmed IPD from 1 January 2003 to 31 December 2005, presenting to a national laboratory-based surveillance programme in South Africa with known survival status. Meningitis was defined as growth of *S. pneumoniae* on cerebrospinal fluid specimen culture (with or without growth from another site) and other-IPD as growth of *S. pneumoniae* from other normally sterile site specimens. Potential predictors for death were evaluated using multiple logistic regression models.
Results: 2251 cases of IPD were reported; 581(26%) with meningitis and 1670 (76%) with other-IPD. The age distribution was: <1 year, 929/2251 (41%); 1-4 years, 854/2251 (38%); 5-14 years, 468/2251 (21%).The overall case fatality rate (CFR) was 22%; 35% (205/581) in meningitis cases and 18% (300/1670) in other-IPD cases (p<0.001). Human immunodeficiency virus (HIV) prevalence was 74% (244/328) amongst meningitis cases and 82% (880/1067 amongst other-IPD cases (p<0.001). On multiple logistic regression analysis, controlling for age group and Pitt bactereamia score, HIV-infected children with meningitis were five times more likely to die compared to HIV-negative children (odds ratio (OR) 5.45, 95% confidence interval (CI) 2.35-12.62). Pitt bacteraemia score >=4 (OR 3.10, 95% CI: 1.22-7.93) and age-group <1 year (OR 2.60, 95% CI: 1.21-5.55) were independent predictors of death in meningitis and other-IPD. Children with other-IPD residing in the Western Cape Province had a significantly lower likelihood of death (OR 0.50, 95% CI 0.32-0.78) compared to children residing in Gauteng and other provinces.
Conclusions: IPD was associated with a high CFR in South African children particularly increasing in HIV-positive children. Universal access to routine use of pneumococcal conjugate vaccine in children as well as increased access to antiretroviral therapy for HIV-positive children should be enhanced.

Appendix 12.5.7: Post Graduate Approval Certificate

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011) 717-2745

Reference: Ms Tania Van Leeve
E-mail: tania.vanleeve@wits.ac.za
18 November 2010
Person No: 0608822F
PAG

Mr PS Nyasulu
School of Public Health
Faculty of Health Sciences
10th Floor
7 York Road, Parktown
2193
South Africa

Dear Mr Nyasulu

Doctor of Philosophy: Approval of Title

We have pleasure in advising that your proposal entitled "*Surveillance of antimicrobial susceptibility patterns among pathogens isolated in public sector hospitals associated with academic institutions, South Africa during a 5 year period 2005-2009.*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 12.5.8: Approval Letter to Access and Use the CDW Data

The Chairperson
HREC
University of the Witwatersrand

Dear Sir/Madam

<u>Permission granted to Peter Nyasulu (PhD Candidate) to obtain data from the Corporate Data
Warehouse (CDW) of the National Health Laboratory Service.</u>

Peter Nyasulu's research project "Surveillance of antimicrobial susceptibility patterns among
pathogens isolated in public sector hospitals associated with academic institutions in South
Africa during a 5 year period 2005-2009" is an important public health issue which falls within
the ambit of the Antimicrobial Resistance Reference Unit (ARRU) of the NHLS. The results of
this study will contribute towards the development of public health policy at a national level
within the Department of Health.

In my capacity as Head of Academic Affairs and QA at the Information Management Unit of the
NHLS, I hereby authorise for Dr Peter Nyasulu to have access to blood culture data during the
period 2005 to 2009 from the NHLS CDW, provided that ethical approval has been obtained
from the University and subject to NHLS conditions guiding the use of and access to CDW data.

Yours sincerely

pp Dr Kerrin Begg
Head, Academic Affairs and QA
Information Management Unit.
National Health Laboratory Service.

Appendix 12.5.9: Ethics Approval Certificate

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49   Dr Peter Nyasulu

CLEARANCE CERTIFICATE   **M10625**

PROJECT   Surveillance of Antimicrobial Susceptibility
                               Patterns among Pathogens Isolated in Public
                               Aector
                               Hospitals Asscoated with Academic Institutions
                               South Africa during a 5 Year Period 2005-2009

INVESTIGATORS   Dr Peter Nyasulu.

DEPARTMENT   School of Public Health

DATE CONSIDERED   25.06.2010

DECISION OF THE COMMITTEE*   Approved unconditionally

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

DATE   28/06/2010   CHAIRPERSON ....
                                          (Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :   Prof HJ Koornhof

DECLARATION OF INVESTIGATOR(S)

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix 12.5.10: Approval Letter for Laboratory Visit at Muhimbili

MUHIMBILI NATIONAL HOSPITAL

Cables:	g"MUHIMBILI"		Postal Address:
Postal Address			P. O. BOX 65000
Telephones:	255-22-2151599		Muhimbili
	255-22-2151369		Dar es Salaam
FAX:	255-22-2150534		Tanzania
			21.03.12
Web	www.mnh.or.tz		

Peter Nyasulu
University of the Witwatersrand
School of Public Health
Division of Epidemiology and Biostatistics
7 York Road, Parktown 2193, South Africa

Ref: Permission to visit Department of Microbiology/Immunology

The permission is granted for you to visit our Department of Medical Microbiology/Immunology, for the purpose of assessing patterns and trends of antimicrobial resistance.

It is our hope that you will benefit from your visit, and we are waiting for your positive inputs, which will enable our department to improve the monitoring system which is in place. This is important because of high population morbidity, needss system for monitoring resistance to be universally standardised to allow for cross country comparison of data.

Sincerely

Eria Daniel
For Executive Director - MNH

MUHIMBILI NATIONAL HOSPITAL
P. O. Box 65002
DAR ES SALAAM

Appendix 12.6: Laboratory Visits Observation Checklist

	Observed parameters	Yes/No
1	Availability of a standard operating procedure	
2	Flow of blood culture specimen in the laboratory	
3	Blood culture procedures: Manual/automated	
4	Susceptibility testing procedures: Manual/automated	
5	MIC break points: CLSI Guidelines in use	
6	Quality Assurance & Control Methods (QA/QC)	
7	Staffing for blood cultures (always same individual/rotational)	
8	Data entry methods: manual/automated	
9	Database: localised or network	
10	Blood culture database: localised or on network linked to CDW	
11	Data transfer : localised entry/automated	

Appendix 12.7: NHLS Laboratory Request Form

Appendix 12.8: MNH Laboratory Request Form

Muhimbili National Hospital
P.O. Box 65000, Dar Es Salaam
Laboratory Test Request Form

Patient name_____ Age_____

Sex _____ Medical record number _____ Ward _____

Ordering Doctor _____ Firm/Clinic _____

Date Ordered _____ Date/Time Collected _____
                                                    (Filled in by phlebotomist)

Brief Patient History _____

Urgency □ EMERGENCY   □ URGENT   □ ROUTINE

Chemistry
- □ Serum Electrolytes (G)
- □ Glucose (G)
- □ Creatinine (G)
- □ Urea Nitrogen (G)
- □ Liver Function Panel (G)
- □ β-hCG Quantitative (G)
- □ Thyroid Stim. Hormone (Y)
- □ Thyroid Panel (Y)
- □ Phenobarbital (Y)
- □ Phenytoin (R)
- □ Gentamycin Peak (Y)
- □ Gentamycin Trough (Y)
- □ Digoxin (Y)
- □ Carbamazepine
- □ Troponin-I (G)
- □ Creatine Kinase (CPK) (G)
- □ Urine Drug Screen (U)

Serology
- □ HIV ½ Antibody Screen (Y)
- □ Hepatitis B Surface Antigen (Y)
- □ Hepatitis C Antibody (Y)
- □ Rubella IgG Antibody (Y)
- □ Rubella IgM Antibody (Y)
- □ Toxoplasma IgG Antibody (Y)
- □ Toxoplasma IgM Antibody (Y)
- □ VDRL (Syphilis) (Y)
- □ Widal Test (Y)
- □ HIV Viral Load (P)
- □ Hepatitis A. B, C Panel (Y)

Hematology /Coagulation/Blood Transfusion
- □ Full Blood Count/Differential (P)
- □ Erythrocyte Sedimentation Rate (P)
- □ CD4/CD8 Panel (P)
- □ Sickle Hemoglobin Screen (P)
- □ Prothrombin Time (B)
- □ Partial Thromboplastin Time Time (B)
- □ Fibrinogen (B)
- □ Blood Film for Malaria Slide
- □ Blood Grouping/Rh (R)
- □ Crossmatch (Compatibility Test for Blood Transfusion)

Microbiology/Parasitology

Specimen _____

Source _____

Test Requested _____

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