**University of Pretoria**

Faculty of Health Sciences

School of Health Systems and Public Health

**Title of the Research Project:**

Microbiological profile of organisms causing bloodstream infections between 2004 and 2016 in a tertiary hospital, Limpopo province, South Africa.

**For the degree:** MSc. Clinical Epidemiology

**Author:** Sizeka Maweya

**Student number:** 95023552

**Contact details:**

Address: Pietersburg Provincial Hospital 18 College Street Polokwane

Tel: 015 287 5010

Fax: None

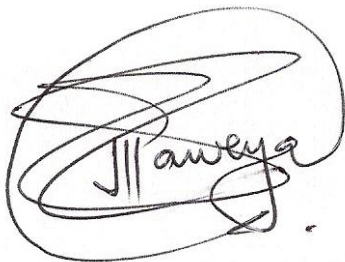Cell: 082 345 9449/079 496 9996

Email: u95023552@tuks.co.za

**Supervisor:** Prof Brendan Girdler-Brown

**Co-supervisor:** Dr. Molebogeng Ruth Lekalakala

**Date: 22/11/2017**

**DECLARATION**

"I, Sizeka Maweya, hereby declare that the mini-dissertation, which I hereby submit for the degree MSc. Clinical Epidemiology at the University of Pretoria, is my own work and has not been submitted by me for a degree at another university".

12/02/2018

_____          _____
**Maweya Sizeka**                           **Date**

**Student Number: 95023552**

# DEDICATION

**I dedicate this work to my son Sizeka Maweya and everybody who contributed to my success.**

# ACKNOWLEDGEMENTS

## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| AAC | Academic Advisory Committee |
| AMR | Antimicrobial Resistance |
| ALOS | Average Length of stay |
| API | Analytical Profile Index |
| ASP | Antimicrobial Stewardship |
| BSI | Blood Stream Infection |
| CDC | Centre for Disease Control |
| CEO | Chief Executive Officer |
| CoNS | Coagulase-Negative Staphylococci |
| CRE | Carbapenem-Resistant Enterobacteriaceae |
| E.coli | Escherichia Coli |
| ESBL | Extended-Spectrum ß-Lactamase |
| HA-BSI | Healthcare Associated Bloodstream Infection |
| HAIs | Healthcare Associated Infections |
| ICU | Intensive Care Unit |
| IPC | Infection Prevention Control |
| KAP | Knowledge Attitude Practice |
| LMIC | Low Middle-Income Countries |
| MDR | Multi-Drug Resistance |
| MIC | Minimum Inhibitory Concentration |
| MRSA | Methicillin Resistant Staphylococcus aureus |
| MSSA | Methicillin Susceptible Staphylococcus aureus |
| NHLS | National Health Laboratory Services |
| NTS | Non-Typhoidal Salmonella |
| OR | Odds Ration |
| SA | South Africa |
| SADC | South African Development Communities |
| SDD | Selective Digestive Decontamination |
| SOD | Selective Oropharyngeal Decontamination |
| UK | United Kingdom |
| UTI | Urinary Tract Infection |
| VRE | Vancomycin-resistant enterococci |
| WHO | World Health Organization |

# ABSTRACT

**Background:** Nosocomial bloodstream infections constitute a significant public health problem and may be an important cause of morbidity and mortality in patients who are hospitalized. The presence of living microorganisms in the blood of a patient is usually indicative of a serious invasive infection requiring antimicrobial therapy. Mortality associated with bloodstream infections may range from 20 to 50% and depends on several factors, including pathogen and host factors. Many septic episodes are nosocomial and may be due to microorganisms with increased antimicrobial resistance.

**Aim:** This study describes the microbiological profile of the organisms, and their resistance to antibiotics, causing bloodstream infections in patients in a tertiary hospital in Limpopo between 2004 to 2006 and 2014 to 2016.

**Methods:** This was a retrospective laboratory-based serial cross-sectional study of 219 cultures in 2004 to 2006 73 of which were positive and 1095 cultures in 2014 to 2016 298 of which were positive. Data, including patient demographics (age, gender), microbial species (as recorded in the blood culture reports) and the antibiograms of isolated microorganisms, was collected and analysed.

**Results:** 371 blood culture results which were culture positive were analysed. Coagulase negative *staphylococci* 190 (51.2%), *Acinetobacter baumannii* 14 (4%), *Klebsiella pneumonia* 44 (11.9%), *Enterococcus spp* 23 (6.2%). *Enterobacter spp* 19 (5.1%), *Staphylococcus aureus* 21 (7.3%), and *Escherichia coli* 14 (3.8%) were predominant. The constitution of bacteria cultures isolated where gram status was known, was gram-positive 262 (70.6%) and gram-negative 106 (28.6%). Among the *S. aureus* isolates, extended-spectrum beta lactamase (ESBL) positivity was 27 (7.3%). The microorganisms exhibited a level of resistance against the following antimicrobials: (colistin, imipenem, linezolid, meropenem and vancomycin).

**Conclusions**: There was an increase in the numbers of tests, and hence the numbers of isolates, between the two study periods. The study demonstrated that there was a less than 3% drug resistance level, against antibiotics tested for, in both time periods. There was no clinically significant change in the resistance levels between the first and second study periods.

**Keywords:** Bloodstream infections, Bacterial isolates, Antimicrobial resistance

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Permission letter from National Health Laboratory Services (NHLS).

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# CHAPTER 1

## INTRODUCTION

### 1.1.    Background to the Research

The presence of living microorganisms in the blood of a patient is usually indicative of a serious invasive infection requiring urgent antimicrobial therapy.

Mortality associated with bloodstream infections may range from 20 to 50% and depends on several factors, including the pathogen and host. Many septic episodes are nosocomial and may be due to microorganisms with increased antimicrobial resistance [1].

Primary bloodstream infection (BSI) is a leading, preventable infectious complication in critically ill patients and has a negative impact on patients' outcome.

Study done in Geneva Switzerland found that BSI represents about 15% of all nosocomial infections and affects approximately 1% of all hospitalized patients. BSI increases the mortality rate, prolongs patient stay in an intensive care unit (ICU), and generates substantial additional costs [2].

Patients with febrile illness have a highest suspicion of blood stream infection (BSI). This therefore becomes the common reason for admission into hospitals in Africa and blood cultures are an important investigation. However, data on the prevalence and causes of community acquired BSI in Africa are scarce and there are no studies from South Africa. Clinical prediction rules for use of blood cultures in Africa is not validated [3].

In the two study periods under review the number of admissions in ICU were 1516 for the study period 2014 to 2016. There were no data available for the number of admissions for the study period of 2004 to 2006.

### 1.2.    Research problem /hypothesis

The aetiology and antimicrobial profile of BSIs continue to change with the evolution of medical care, particularly among hospitalized patients who require intensive care support and antimicrobial treatment.

BSIs remain one of the most frequent infection despite the advances in therapy and supportive care. Bloodstream infections are common in hospitalized patients and inadequate treatment results in mortality and an increased number of resistant organisms. Surveillance studies provided important information that allows for the identification of trends in pathogen incidence and antimicrobial resistance.

The study was initiated because of the limited published data on the prevalence and microbiological profile of organisms causing BSI in patients admitted at the Pietersburg Provincial Hospital which is a tertiary hospital in the Limpopo province.

**Hypothesis**

$H_1$

There was a change in the patterns of infectious agents isolated at Intensive Care Unit of the Pietersburg Provincial Hospital over the study periods between 2004-2006 and 2014-2016.

$H_o$

There was no change in the patterns of infectious agents isolated at the Intensive Care Unit of the Pietersburg Provincial Hospital over the periods between 2004-2006 and 2014-2016.


**1.3.    Justification for the research**

It is important for individual facilities and clinicians to have local data as the prevalence of organisms and their antimicrobial profile tend to vary from facility to facility and even unit to unit.

Such information is essential to guide appropriate management of such infections, taking into consideration the reported prevalent microbial pathogens and their susceptibility profile at a given time.

The study findings will provide valuable baseline information required for the implementation of an antibiotic stewardship initiative; inform local hospital-based guidelines and inform policy development to optimize patient care management.

The two study periods were studied because we wanted to compare as to, were there changes in the type of pathogens isolated ten years apart. Pietersburg hospital was functioning as a regional hospital in the years 2004 to 2006 referring most of the patients to Dr George Mukhari hospital in Gauteng. In the years 2014 to 2016 it was and it still is functioning as the only tertiary hospital in Limpopo and complemented by an increase number of specialists and the presence of a microbiologist in the National Health Laboratory Services (NHLS). We needed also to determine the antimicrobial patterns that were 10 years apart with the expectation that the study findings would detect an exponential rise in resistance on organism- drug match in the ten-year interval. The three years was to check if there would be rapid change year on year. The significant change was evident in year 2014- 2016 as reported.

## 1.4. Methodology

### 1.4.1. Aim of the study

The aim of this study was to describe the prevalent bacterial pathogens isolated from the blood culture specimens received from the Pietersburg Provincial Hospital and to review the antimicrobial profiles thereof. The study findings will provide information on the trends and distribution of BSI pathogens in the Pietersburg Provincial Hospital and provide valuable information for the implementation of antibiotic stewardship initiatives; provide data that may inform development of guidelines  or review; assist in the monitoring of infection control practices and, importantly, to optimize patient care management [4].

### 1.4.2. Laboratory methods used to test blood culture isolates

A causative agent was used to determine the susceptibility patterns, which guided the selection of antimicrobials agents. After collection by the clinicians, blood culture bottles were incubated in instruments that were generally referred to as the 'blood culture' machine.

The bottle remains incubated for a defined period (five days in this case) before releasing them as no growth. In cases where the bottles are "flagged positive" – i.e. there was a detection of growth by the machine, the bottle was pulled out and

processed further and the peak time to recover an organism was usually between 48 to 72 hours of incubation.

**2004-2006:**

The BD BACTEC™ Blood Culture Media – BD was used for incubation of the bottles; however, the identification was done with Analytical Profile Index (API) which is a manual identification of organisms with a limited spectrum.

**2014- 2016:**

The same BD BACTEC™ Blood Culture Media – BD was used for incubation of the culture bottles. However, identification and susceptibility testing were done using Microscan® which is a semi-automated instrument.

This instrument is able to produce both the identification and susceptibility in less than 24-hour process.

### 1.4.3. Objectives

The objectives of the study were to:

- Determine which organisms were isolated in the ICU of the Pietersburg Provincial Hospital in each of the two study periods under review.

- Compare prevalence (and drug resistance patterns) between the two time periods.

- Determine the rate of extended spectrum of beta lactamase (ESBL) and the emergence of carbapenem-resistant *enterobacteriaceae* (CRE) amongst the prevalent *enterobacteriaceae*.

- Determine the proportion of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci.

- Determine the demographic profile of patients who had BSIs (age, gender, main pathology, specimen type).

## 1.5.    Outline of the report

In this chapter (Chapter One), the introduction to the study is presented and the background and problem statement of the study is set out. The research question and the aims and objectives of the study are explicated, as well as the importance and benefits of the study. A review of the current literature and the limitations that were observed in the available literature are discussed in Chapter 2.

The methodology that was used during the execution of this study is described in Chapter 3. Analysis of the data/results and findings is presented in Chapter 4. Discussion of the study findings is dealt with in Chapter 5. Conclusions and implications of the study is dealt with in Chapter 6.

| Chapter 1 | • Introdution and Background |
| Chapter 2 | • Literature Review |
| Chapter 3 | • Methodology |
| Chapter 4 | • Analysis of data/Results/findings |
| Chapter 5 | • Discussion |
| Chapter 6 | • Conclusions and implications |

Figure 1.1: Outline of the Dissertation

### 1.6. Definitions of terms

#### 1.6.1. Antimicrobial stewardship

**Antimicrobial stewardship** (AMS): "antimicrobial stewardship involves selecting an appropriate drug and optimizing its dose and duration to cure an infection while minimizing toxicity and conditions for selection of resistant bacterial strains" [5].

#### 1.6.2. Minimum inhibitory concentration

**Minimum inhibitory concentration** (**MIC**): "is the lowest concentration of the antimicrobial that will inhibit the visible growth of the microorganism after overnight incubation, and minimum bactericidal concentrations (MICs) as the lowest concentration of antimicrobial that will prevent the growth of microorganism after subculture on to antibiotic-free medium" [6].

#### 1.6.3. Multidrug resistance

"Acquired non-susceptibility to at least one agent in three or more antimicrobial categories" [7].

CHAPTER 2

**THE LITERATURE REVIEW**

**2.1. Introduction**

Chapter 1 set out the background to the study, the research question and the hypothesis. The aims, objectives and general outline of the dissertation were also presented in Chapter One. In this chapter, the literature that is relevant to the study is reviewed and discussed in detail. The search strategy used involved employing the word "bloodstream infections" in the following search engines: Google Scholar, World Cat, PubMed, Science Direct, Cochrane and Google. The referencing software used comes from Mendeley.

**2.1.1. Background**

Study done in South Africa in Groote Schuur Cape Town found that BSIs cause considerable morbidity and mortality and it is estimated that 10 - 13% of community-onset BSIs are fatal [8].

Studies done in Malawi, Kenya and Tanzania of the patients admitted in these hospitals of Africa estimated that 13.5% of adults and 8.2% of children had community-acquired BSIs, indicating these are a common cause of illness and account for a substantial proportion of all healthcare admissions. Rapid diagnosis, identification of the causative bacterial pathogens and appropriate treatment are essential in the mitigation of morbidity and mortality associated with BSIs [9].

BSIs due to bacterial pathogens affect over 200,000 individuals annually in the United States alone. BSIs are tremendously important and have caused a great deal of morbidity and mortality worldwide. The attributable mortality of BSI is approximately 27% and a recently published national vital statistics report in that the country has documented an increase in age-adjusted death rates due to septicaemia from 4.2 per 100,000 in 1980 to 13.2 per 100,000 in 1992 respectively [10].

Bloodstream infection is a life-threatening condition that may be complicated by septic shock and death.

Mortality due to septic shock can be as high as 60% despite the instituted treatment. A better understanding of the spectrum of pathogens causing BSI is crucial for prompt management of patients, as antimicrobial therapy greatly influences the outcome of patients with BSI [11].

The risk of BSI is inherently higher in critically ill patients due to underlying co-morbidities and more invasive medical procedures. An analysis by ICU status (including adult, paediatrics and neonatal) effectively demonstrates this increased risk [12].

### 2.1.2. Incidence

A study undertaken in hospitals in the United States found that 87% of BSIs were mono-microbial. Gram-positive organisms were found to be responsible for 65% of these infections, while gram-negative organisms caused 25% of these infections. The crude mortality rate was found to be in the region of 27%. The same study found that the most common organisms causing BSIs were coagulase-negative Staphylococci (CoNS) (31% of isolates), *Staphylococcus aureus* (20%), enterococci (9%) and *Candida* species (9%) [4].

The mean interval between admission and infection was 13 days for infection with *Escherichia coli*, 16 days for *S. aureus*, 22 days for *Candida* species and *Klebsiella* species, 23 days for enterococci, and 26 days for Acinetobacter species. CoNS, *Pseudomonas* species, *Enterobacter* species*, Serratia* species, and *Acinetobacter* species were more likely to cause infections in patients in intensive care units.

Patients who are neutropenic, infections with *Candida* species, enterococci, and viridans group Streptococci were more common [4].

Population studies done in the past have identified that some cases can be poly microbial as per a study done in Canada where eight cases (3.2 per 100 000) were found to be poly-microbial.

The most common isolates in that study were *E. coli, S. aureus* and *Klebsiella* spp. with rates of 19, 9.7 and 8.9 per 100 000 population, respectively [13].

The SENTRY study conducted for the surveillance program revealed that *Staphylococcus aureus* was found to be the most common cause of BSI, skin and soft tissue infections and pneumonia [14]. A high incidence of nosocomial infections (NI) occurs in intensive care units, and these infections are becoming one of the most important problems in ICUs.

It is well known that these infections are a major cause of morbidity and mortality in critically ill patients and are associated with increases in the length of stay and excessive hospital costs [15].

Richards et al., (1999), based on their study done on the patients in ICU by infection site, indicated that 87% of primary BSIs were associated with central lines. 86% of nosocomial pneumonia was associated with mechanical ventilation. 95% of urinary tract infections were associated with urinary catheters. The same study found that coagulase-negative Staphylococci (36%) was the most common BSI isolated, followed by enterococci (16%) and *Staphylococcus aureus* (13%). 12% of bloodstream isolates were fungi. The most frequent isolates from pneumonia were Gram-negative aerobic organisms (64%). *Pseudomonas aeruginosa* (21%) was the most frequently isolated of these. *S. aureus* (20%) was isolated with similar frequency [15].


### 2.1.3. Prevalence

Nosocomial infections are seen far more often in ICUs than in normal wards due to the immuno-suppressed state of many ICU patients and the continuous use of invasive diagnostic and therapeutic procedures. Most of these infections are of endogenous origin [16].

*Staphylococcus aureus* is one of the most important human pathogen and has, over the past several decades, been a leading cause of hospital and community-acquired infections. It has been associated with a variety of clinical infections including septicaemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis and post-surgical toxic shock syndrome with substantial rates of morbidity and mortality [17].

One of the reasons for the success of this human pathogen is its great variability, occurring at different periods and places with diverse clonal types and antibiotic resistance patterns within regions and countries.

Although infections caused by antibiotic-resistant *S. aureus* bring about serious problems in the general population, such infections can be particularly devastating for the very young, the elderly and the immunocompromised [17].

*Staphylococcus aureus* is the primary cause of nosocomial infection in the United States. In a study conducted in New York City, Methicillin Resistant *Staphylococcus aureus* (MRSA) accounted for 30% of nosocomial infections and 50% of associated deaths [18].

Healthcare-associated infections (HAIs) are the leading cause of significant morbidity and mortality in patients receiving healthcare. The costs (direct and indirect) of these infections deplete the limited financial resources allocated to healthcare delivery. It is estimated that one in seven patients entering South African hospitals is at high risk of acquiring a HAI. Of these infections, lower respiratory tract infections, bloodstream infections and post-surgical infections account for the majority (about 80%) of HAIs [19].

The risk of BSI is inherently higher in critically ill patients due to underlying co-morbidities and more invasive medical procedures. An analysis by ICU status (includes adult, paediatrics and neonatal) effectively demonstrates the increased risk. *Staphylococcus aureus* remains a major causative organism of healthcare-associated bloodstream infection (HA-BSI), responsible for 17% of all HA-BSI episodes (including polymicrobial episodes where *S. aureus* was one of the organisms isolated) [12].

Bloodstream infections are frequent and can usually cause high case-fatality rates. Urgent antibiotic treatment can save the lives of patients. However, antibiotic resistance can render antibiotic therapy futile. A study conducted in Zanzibar found that the most frequently isolated microbes are *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter* spp*. and Staphylococcus aureus.* some of which were community acquired. This was a very worrying factor because it rendered most of the antimicrobials used at the time resistant [20]. Worldwide transmission of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) and their subset producing carbapenems is alarming. However, limited data on the prevalence of such strains in patients from Sub-Saharan Africa is currently available.

A study done in Banako in Mali in two teaching hospitals, which are at the top of the health care pyramid in that country, showed that 77 patients had an E-BSI and as many as 48 (62.3%) were infected with an ESBL-E. ESBL-E BSI were associated with a previous hospitalization and were more frequent in hospital-acquired episodes. Among the 82 isolated Enterobacteriaceae, 58.5% were ESBL-E (*Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae*) [21].

The prevalence of Gram-positive pathogens causing bacteraemia has increased over the past 20 years. This is mainly due to the increase in the coagulase-negative *Staphylococci* (CoNS) and enterococci.

This increase can be attributed to the selective pressure exerted by the use of broad-spectrum antibiotics, such as the third-generation cephalosporins and fluoroquinolones, which are generally more potent against Gram-negative than Gram-positive bacteria. This increase can also be attributed to an increased use of invasive devices [22].

## 2.1.4. Working Classification of bloodstream pathogens

```
                         Gram Positive Bacteria
                    ┌──────────────┴───────────────┐
                  Cocci                           Rods
            ┌───────┴────────┐              ┌───────┴────────┐
         Aerobic         Anaerobic       Aerobic         Anaerobic
            │                │              │                │
     Staphylococci    Peptostreptococci   Bacillus      Actinomyces
     Streptococci                         Listeria      Clostridium
```

```
                         Gram Negative Bacteria
                    ┌──────────────┴───────────────┐
                  Cocci                           Rods
            ┌───────┴────────┐              ┌───────┴────────┐
         Aerobic         Anaerobic       Aerobic         Anaerobic
         Facultative        │              │                │
            │           Veillonella   Enterobactericcea  Pseudomonas  Bacteroidses
         Neiseria                          │             Vibrio       Fusobacterium
         Moraxella                   ┌──────┴──────┐      Hemophilus
                                  Lactose      Non-lactose
                                  Fermenters   Fermenters
                                  E. Coli      Salmonella
```

Figure 2.1: Classification of Gram-positive and Gram-negative microorganisms

A study done over a period of 29 years found that *Staphylococcus aureus*, coagulase-negative *Staphylococci* (CoNS), *Enterococcus* species, *Clostridium difficile* and other anaerobes significantly increased, whereas *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* species, *Enterobacter* species, and other *Streptococci* significantly decreased in relative proportion in frequency of pathogens [23].

The rapid expansion of ESBL resistance among common Gram-negative pathogens, and the emergence of MRSA, highlight the growing challenge of BSIs that are effectively impossible to treat in resource-limited settings [24].

CoNS is the most common of pathogens within all clinical services, with the exception of obstetrics, where *Escherichia coli* is most common.

Methicillin resistance is detected most commonly; *Staphyloccocus aureus* and CoNS isolates are the most common isolates in a hospital setup. Vancomycin resistance in enterococci is species-dependent, in particular to the *Enterococcus faecalis* strains, while *Enterococcocus faecium* isolates also display resistance to vancomycin [25].

Critically ill patients are particularly vulnerable to hospital-acquired infections. These hospital infections are two to seven times more common in ICUs and account for approximately half of all hospital-acquired BSI. BSI is more common in patients who have had surgery, who are immunocompromised, who usually develop multiorgan dysfunction, who require mechanical ventilation or renal replacement therapy and who have greater illness severity on ICU admission. Some of the critically ill patients may be genetically predisposed to developing BSI and dying in hospital. Thus, BSI may be a marker of illness severity and pre-morbid condition as well as a direct contributor to adverse outcome [26].

There is an increasing isolation in the frequency of Gram-positive pathogens in severe ICU infections which has resulted in greater usage of vancomycin. This may account for the rising incidence of vancomycin-resistant enterococci [22].

## 2.1.5. Antimicrobial susceptibilities of gram-positive pathogens

Antimicrobial agents were tested against the four most prevalent causes of Gram-positive BSI (*S. aureus*, CoNS, enterococci, and *S. pneumoniae*) in the United States and Canada. In both of these countries these microorganisms accounted for approximately 50% (51% in the United States and 49% in Canada) of all bacteraemia episodes [10]. Studies undertaken in Belgrade found that antibiotic resistance of CoNS was highest when compared to beta-lactam antibiotics, macrolides, and tetracyclines. Tigecycline, linezolid, and vancomycin produced the highest activities against CoNS in *in-vitro* conditions, and consumption of linezolid and tigecycline increased during the period that the study was conducted.

Novel antimicrobial agents are still unavailable and/or too expensive in developing countries. However, inappropriate use of the available antibiotics may lead to the rapid development of resistant strains in the near future [27].

Most of the pathogens isolated from blood cultures show high rates of resistance to the most commonly used antibiotics, that is, antibiotics used to treat bacterial infections. Rational use of antibiotics should be practiced at all times [28].

The adverse outcomes of nosocomial infections caused by resistant pathogens (MRSA and VRE) include increased hospital mortality, increased length of stay in hospital and intensive care unit (ICU) costs also increase.

Two meta-analyses conducted in the USA demonstrated that bacteraemia caused by MRSA is associated with significantly higher mortality rates than bacteraemia caused by methicillin-susceptible *Staphylococcus aureus* [29].

Inadequate antimicrobial therapy is relatively common, with studies generally reporting an incidence of inappriate antibiotic therapy. Inadequate antimicrobial treatment is an important factor in the emergence of infections caused by antibiotic-resistant bacteria. Factors associated with administration of inadequate antibiotic therapy include the presence of resistant pathogens, prior antibiotic administration and invasive procedures. Other factors that may contribute to inadequate antimicrobial treatment include the use of broad-spectrum antibiotics, prolonged hospital stay and prolonged mechanical ventilation. All of the above are also attributable to the Gram-positive nosocomial pathogens [24].

Studies done in the past have demonstrated that there is higher mortality, prolonged length of hospital stay and higher costs associated with methicillin-resistant *Staphylococcus aureus* infections when compared to methicillin-susceptible *Staphylococcus aureus* (MSSA) infections. Similarly, vancomycin-resistant enterococci BSIs have a negative impact on mortality, length of hospital stay and costs when compared to infections due to vancomycin-susceptible enterococci. These challenges demonstrate that distinctive prophylactic and therapeutic approaches have to be undertaken to successfully prevent the clinical consequences of antibiotic resistance in Gram-positive bacteria [30].

Infections with Gram-positive rods are rare in comparison to those caused by Gram-positive cocci or Gram-negative rods. Gram-positive rods do not often figure in antibiotic susceptibility studies and very few, if any, large-scale or controlled clinical studies involving these organisms are available. Only anecdotal experience exists for many antimicrobials [31].

It has also emerged that serious infections caused by Gram-positive pathogens are becoming increasingly difficult to treat because of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae*. The more recent emergence of vancomycin-intermediate and -resistant MRSA (VISA and VRSA) has further compromised treatment options. Resistance to, and clinical failures with newer antimicrobial agents such as linezolid, have also emerged.

There is a clinical need for new antimicrobial agents that have suitable pharmacokinetic properties and safety profiles with activity against the Gram-positive pathogens [32].

There is a perception that multidrug resistant bacteria (MDR) can also be due to colonization. Studies conducted in Europe found a high rate of MDR colonization among patients previously hospitalized in the (sub) tropics, whereas the figures for patients from the temperate regions were low. Furthermore, a study using multivariate analysis identified several independent risk factors, which were: destination, invasive procedure or antimicrobial use abroad, age <6 years, visiting friends and relatives travel or foreign residence, direct inter hospital transfer, and short time since hospitalization [33].

Multidrug-resistance among Gram-positive genera is increasing globally, particularly in pathogens such as penicillin-resistant pneumococci, methicillin-resistant *S. aureus* (MRSA) and ampicillin-resistant enterococci, all of which are now resistant to glycopeptides. These developments pose a serious threat to the efficacy of available antimicrobials [34].

Methicillin-resistant *Staphylococcus aureus* strains have become a major problem in many countries, both affluent and poor. A study conducted by Finch found that prevalence varies markedly from country to country, with very high levels of *S. aureus* isolates reported in the Far East, while lower prevalent levels were reported in countries such as Germany, the Netherlands and Switzerland [35].

Any bacteria can develop antimicrobial resistance (AMR), but still maintain its susceptibility to many others, allowing for successful treatment in clinical settings. A selected group of bacteria has been described by the acronym of ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) [36]. *Staphylococcus aureus* is the most significant cause of Gram-positive bacteraemia in the developed world, where the incidence varies between10 to 30 per 100 000 person-years. Methicillin resistance is the hallmark of antimicrobial resistance in both *S. areus* and CoNS, and can be regarded an indicator for multidrug resistance [36].

### 2.1.6. Antimicrobial susceptibilities of Gram-negative pathogens

The treatment of bacterial infections is increasingly becoming more complicated due to bacteria developing resistance against antimicrobials.

The principal mechanism of action of the antimicrobials is usually categorized by their mechanisms of action. Mechanisms of action include the following: interference with cell wall synthesis (e.g., β-lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole) and disruption of bacterial membrane structure (polymyxins and daptomycin).

Bacteria may be resistant to greater/equals to class of antimicrobial agents, or it may acquire resistance by *de novo* mutation or via the acquisition of resistance genes from other organisms [37].

Gram-negative pathogens producing extended spectrum of β-lactamses are now common and are associated with high rates of inadequate empirical treatment and mortality. In addition, carbapenem resistance is increasing, leaving clinicians with limited therapeutic options. Better knowledge of local epidemiology can help to optimize therapies [38]. Enterobacteriaceae emergence and spread is complicating the treatment of serious nosocomial infections which is threatening the current available antimicrobial agents. Twenty percent of *Klebsiella pneumoniae* infections and 31% of *Enterobacter* spp infections in intensive care units involves strains that are no longer susceptible to third-generation cephalosporins [39].

Pandrug resistance of the Gram-negative pathogens such *as P. aeruginosa, A. baumannii or K. pneumoniae* is relatively rare but there is emerging evidence that if care to avoid their resistance is not carefully taken there will be challenges in the future [40].

A study done in South Africa found that there was compelling evidence to conclude that the overall resistance rate to some pathogens (MIC > 8 mg/l) was 56.2%, and high-level resistance (MIC > 1024 mg/l) occurred in 24,0% of the total [41].

Studies performed in the Congo found that Gram-negative organisms mostly isolated were Enterobacteriaceae, particularly *E. coli.* Discrepancies were discovered based on the fact that what was observed was not in line with what was reported in the earlier years [42].

Antibiotic therapy in an ICU is sometimes given empirically while awaiting blood culture results. However, empirical therapy should be designed with regard to the bacterial epidemiology and the aim should be to optimize outcomes while attempting to reduce the potential for resistance development. The antimicrobial therapy for resistant pathogens includes the following carbapenems, ertapenem for ESBL's, cefepime, piperacillin/tazobactam and, on occasion, the Gram-negative quinolones, ciprofloxacin and levofloxacin.

Consideration should be given to the possibility of 'collateral damage', where overuse of an antibiotic predisposes to multi-drug resistance. The use of antibiotics should be limited, where possible, to those organisms that are pathogens and not colonizers and should be discontinued if blood culture results are available where sepsis is not confirmed or there is rapid resolution of clinical indicators of sepsis [43].

Carbapenems have always been the first-choice drug prescribed to patients in ICU where Enterobacteriaceae was involved, even though it has not been adequately evaluated as the causative organism. The treatment of ICU-acquired infections due to carbapenem-resistant Gram-negative bacteria is currently scarce. There are recent reports emphasizing the spread of colistin resistance in environments with a high volume of polymyxins use. These reports have elicited a major concern [44].

Sometimes it is unavoidable to prescribe antimicrobials empirically. If an occasion warrants the use of the antibiotic for the Gram-negative organisms, one should never use any antimicrobial that has been used within the last 12 months because resistant to such an antimicrobial might have occurred [45].

Studies undertaken in the past have indicated that elderly patients, severe underlying illness and ICU admission are risk factors for carbapenem resistance *Enterobacteriaceae* (CRE) bacteraemia. High rates of carbapenem resistance *Acinetobacter baumannii* from the bloodstream presents a huge dilemma to clinicians regarding the choice of empirical antibiotic treatment, as inappropriate empirical treatment can lead to high mortality rates [46].

In resource-constrained settings Infections caused by some superbugs, such as carbapenem resistance and extensively drug-resistant Gram-negative pathogens, pose a great challenge. The lack of last resort antibiotics such as colistin and tigecycline makes treatment difficult [46]. It must be noted that a delay in initiating empiric antibiotics will result in the development of resistance because resistance is time bound. Appropriate antimicrobials must be started 24 to 48 hours post availability of blood culture results [47].

Studies done in the past indicate that it is not rare to develop colistin resistance.

Cognisance must be taken of the fact that patients who stayed longer in ICU and hence received longer periods of colistin treatment are more likely to develop colistin resistance, which is regarded as the superbug treatment option [48].

Nosocomial infections caused by *P. aeruginosa* and *A. baumannii* respond very well to colistin. However, a colistin side-effect is nephrotoxicity which can also be attributed to other comorbid conditions, especially for patients admitted in ICU [49].

### 2.1.7. Antimicrobial stewardship

The emergence of antimicrobial resistance has prompted countries, through the guidance of World Health Organization (WHO), to formulate the legislative framework on antimicrobial resistance. The Centre for Disease Control (CDC) formulated the 12 steps as core elements to guide hospitals on the antibiotic stewardship programs.

Prevent infection: vaccinate and get the catheters out. diagnose and treat infection effectively by targeting the pathogens, get access to the experts. Emphasis was also placed the wise use of antimicrobials. Practice antimicrobial control and also use local data as a guide to intervention. Treat infection, not contaminations and treat infection, not colonisations. Healthcare workers must also be taught to know when to say "no" to vancomycin. Stop treatment when infection is cured or unlikely to prevent transmission. Isolate the pathogen and break the chain of contagion [50].

Antimicrobial use is the key driver of resistance and the selective pressure comes from overuse in many parts of the world. The WHO has also noticed that antibiotics are also used to treat minor infections and that misuse also stem from lack of access to appropriate treatment. Inadequate use of antimicrobials can also be driven by a lack of financial support to complete the treatment courses, in some instances. Hence, one of the recommendations made by the WHO was that countries should be encouraged to develop sustainable systems in order to detect resistant pathogens and to monitor volumes and patterns of antimicrobials use. In so doing the impact of control measures should be detected timeously [51]. South Africa is guided by the constitution which states that:

"Guided by the substantive content of all laws and policies through its Bill of Rights. The Constitution provides for health policy and practices that respond to the needs of South Africans. In terms of Section 27 of the Constitution access to health care in itself is a basic human right.

"All reasonable measures must be taken to ensure that this right is protected, promoted, and fulfilled within the limits of available resources". The Health Act further states: establishment of "a system of co-operative governance and management of health services, within national guidelines, norms and standards, in which each province, municipality and health district must address questions of health policy and delivery of quality health care services".

The Department of Health, through its guidance, has mandated hospitals to formulate antimicrobial stewardship interventions.

The hospitals must put in place, as part of its strategic framework, antimicrobial stewardship (AMS) ward rounds, which have been shown to reduce the prescription of antibiotics in South Africa without affecting patient safety [52].

No defined acceptable norms for antimicrobial resistance levels were found in the literature, either from CDC or WHO; and hence there is no literature review section on this topic.

# CHAPTER 3

## METHODOLOGY

### 3.1.  Introduction

Chapter 1 dealt with the background to; the rationale for; and the aims and objectives of the study. In Chapter 2, literature pertaining to the profile of microorganisms which cause nosocomial infections, and their susceptibility, was dealt with.

In Chapter 2 we also learned about the rational for the use of the empirical antimicrobials while awaiting blood cultures.

In this chapter, we are going to learn about the methodology used and how the study was conducted in order to arrive to the findings.

In this chapter, the research methodology is set out in terms of the study design, the study setting and the procedure.

### 3.2.  Justification of the methodology

It was important for our facility (Pietersburg Provincial Hospital) and our clinicians that this study used local data, as the organism prevalence and their antimicrobial profile tend to vary from facility to facility and even unit to unit. Local information is essential to guide the appropriate management of BSIs, taking into consideration the reported prevalent microbial pathogens and their susceptibility profile at a given time.

To achieve this, a cross-sectional, laboratory based study comparing two study periods (2004-2006 and 2014- 2016) was undertaken to determine the prevalent bacterial pathogens causing BSIs and the antimicrobial profile thereof.

The local data used was drawn from the NHLS and the approach to the methodology assisted the researcher to compare the two-time periods in a way that would inform best practice and an evidence-based approach.

### 3.3. Research procedures

### 3.3.1. Study setting

The study was conducted at the Pietersburg Provincial Hospital, which is a 500-bed tertiary hospital. Most of the culture results that were analysed were drawn from the adult ICU, which is a 12-bed, multidisciplinary unit. Specimens or tests were also collected from the hospital's paediatrics ICU, which has 6 beds.

Pietersburg Provincial Hospital is the biggest tertiary hospital in the Limpopo province, with 18 clinical disciplines. It is a referral hospital for the 5 regional hospitals in the province.

The staff complement in the adult ICU consisted of 1 operational manager, 32 professional nurses and 4 enrolled nurses. Specialist medical care was provided on an *ad hoc* basis, since there was no full-time specialist appointed to the ICU.

The paediatrics ICU had 2 pulmonologists, 1 operational manager, 7 professional nurses and 6 enrolled nurses. The National Health Laboratory Services (NHLS), who provided the results, were operating inside the hospital premises with 7 technicians, 5 technologists and 1 microbiologist.

### 3.3.2. Study isolates from blood cultures

Information from all the patients who were admitted in the ICU between January 2004 to 2006 December and January 2014 to 2016 December with available laboratory records of their positive blood cultures was drawn.

### Inclusion criteria

All patients who were admitted to the ICU of this tertiary hospital on whom blood cultures were performed in the defined study period were included in the study.

**Exclusion criteria:**

Patients with blood culture investigations from NHLS who were not admitted to the ICU during the study period were excluded.

Blood culture results that did not yield significant (non-pathogenic) organisms (skin commensals) were excluded.

### 3.3.3. Sampling

**Sampling method:**

All positive the blood culture results of patients who met the inclusion criterion were included in the study. The raw data of blood culture results was provided in an Excel spreadsheet by the Polokwane Microbiology Division of the National Health Laboratory Services for analysis.

**Sampling size:**

All blood culture isolates tested during two three-year periods over an interval of 10 years (between 2004 – 2006 and 2014 - 2016) were analysed.

It was anticipated that a total of 372 blood culture results per each 3-year period from 2004 – 2006 and 2014 - 2016 would be available for analysis when considering the average blood cultures positivity rate of 20 -25% (an estimation of positive blood culture results was 25% of 423 = 105.75 pulled from the ICU proportion). Relative proportions were used to compare the prevalence of drug resistances in the two study periods.

Power and sample size software was used to estimate the required sample size on the assumption that a relative proportion of 2 or greater would be of practical interest. The power of 80% was used to detect a difference; the required sample size was 200 in each of the two study periods.

In fact, it was expected that there would be more than 200 blood culture positive results with susceptibility testing results from both time periods (all organisms isolated). Resistance was defined as, resistance to at least the usual first line drugs for a particular organism. iI then present it was deemed to be present.

### 3.3.4. Measurements

Data, including patient demographics (age, gender), microbial species (as recorded in the blood culture reports) and the antibiograms of isolated microorganisms, was collected and analysed. The prevalence, trends, ratios and percentages of variables measured was presented using tables, graphs, charts and figures in this chapter.

The groups of interest made up of the organisms that constituted resistance was defined as follows:

For all ESBL organisms – first line would be carbapenems (imipenem or meropenem)

CRE – Colistin

MRSA – Vancomysin

VRE - Linezolid

### 3.3.5. Pilot Study

This was a retrospective study involving archived data, therefore, there was no need to undertake a pilot the study, since the official reports of patients used were those that had been previously released.

### 3.4.    Data analysis plan

No additional data were collected. Only the data provided by the NHLS in a form of an ESpreadsheet was analysed.

These data were entered into Epidata and saved as a Stata file for analysis in Stata version 13 from StataCorp LP (Serial number 301306259987).

Summary measures included proportions for binary variables and ratios for categorical variables with more than 2 outcome states.

Chi square tests or Fisher's exact tests, was used to determine whether any changes in antibiotic resistance were statistically significant (alpha = 0.05).

## 3.5.   Data management

Data storage was electronic based, compatible with the format of the data collected. The data was stored in Dropbox, iCloud and Google Cloud for a period of ten years. The data were also routinely stored by the NHLS.

## 3.6.   Ethical considerations

Approval of the research protocol was sought from the Academic Advisory Committee (AAC) and the Research Ethics Committee of Faculty of Health Sciences, University of Pretoria and NHLS. Confidentiality was maintained as no names were included in the extracted data and no potential harm was envisaged.

A potential conflict of interest might be that the researcher works in the same hospital in which the study was conducted and formed part of the management team and the team of clinicians. The NHLS gave permission to conduct the study, however, the actual data extraction was done once the study had been approved by the university's ethics committee.

## 3.7.   Conclusion

All processes for performing the study were followed, including ethics approval from both the Academic Advisory Committee and the Faculty of Health Sciences Research Ethics Committee (**Ethics Reference No: 332/2017).**
The methods, as guided by the researcher's supervisors, were adhered too.

# CHAPTER 4

## PRESENTATION AND INTERPRETATION OF THE RESULTS

### 4.1 Introduction

The aim of this study was to describe the prevalent bacterial pathogens isolated from the blood culture specimens received from Pietersburg Provincial Hospital and to review the antimicrobial profile thereof. This chapter outlines how the results correlated with the data that were analysed, that is, whether the trends found in our setting were in line with those found in other centres, as per the following objectives:

- To determine the demographic profile of patients who had BSIs (age, gender, main pathology, specimen type).
- To determine which organisms were isolated in each of the two comparisons periods.
- To compare drug resistance patterns between the two study periods.
- To determine the rate of ESBL and the emergence of CRE amongst the prevalent Enterobacteriaceae.
- To determine the proportion of MRSA and vancomycin-resistant enterococci.

### 4.2. Patient Demographics

A total of 371 patient blood culture records were reviewed during the study period. Of these, 234 (63%) were drawn from males, while only 123 (33%) were drawn from females. Fifteen (4%) were drawn from patient of unknown sex.

Figure 4.1 shows the gender proportion/ distribution of the two study periods. There was a significant increase in the proportion of records drawn from males from 48% in 2004-2006 to 67% in 2014-2016 ($p<0.001$; chi square test).
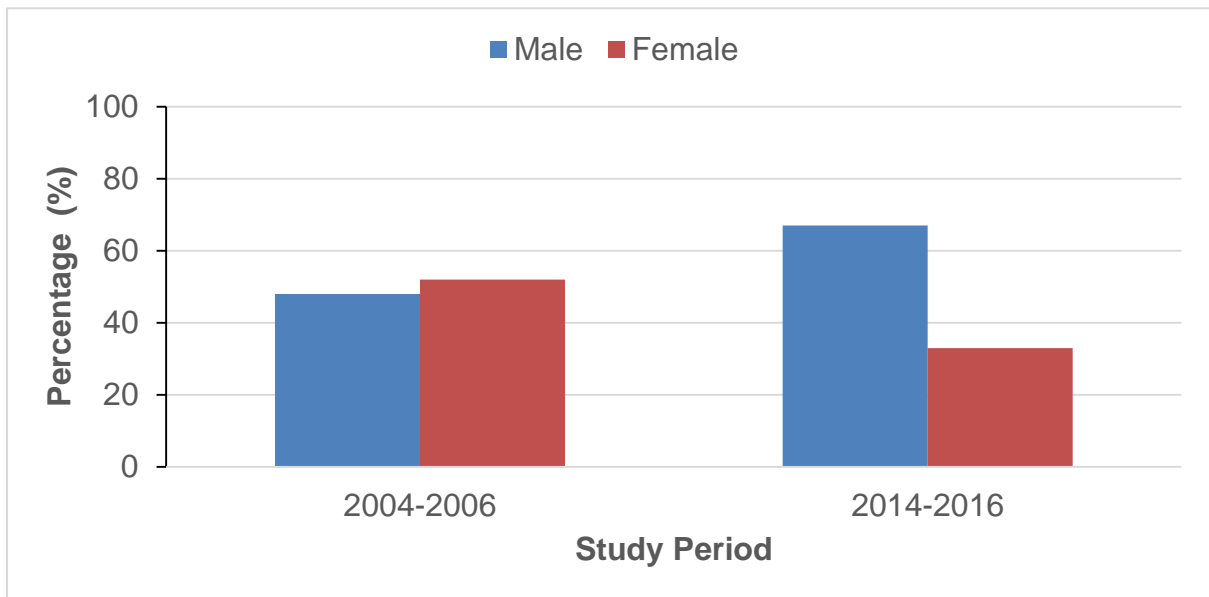
Figure 4.1: Gender proportion/ distribution of the two study periods

The mean age of the patients was 28.8 ±21.9 years ranging from 1 month to 90 years old. Overall, greater proportions (20%) of the patients were aged less than 5 years of age, followed by those in the age group (16%) 30-39 years and (15%) were in the 20-29 years age group. The age distribution per study period is illustrated in Figure 4.2. There was a significant association between period of study and age ($p < 0.001$; chi square test).

This significant change was mainly due to the increased number of bloods cultured from the under-fives age group.
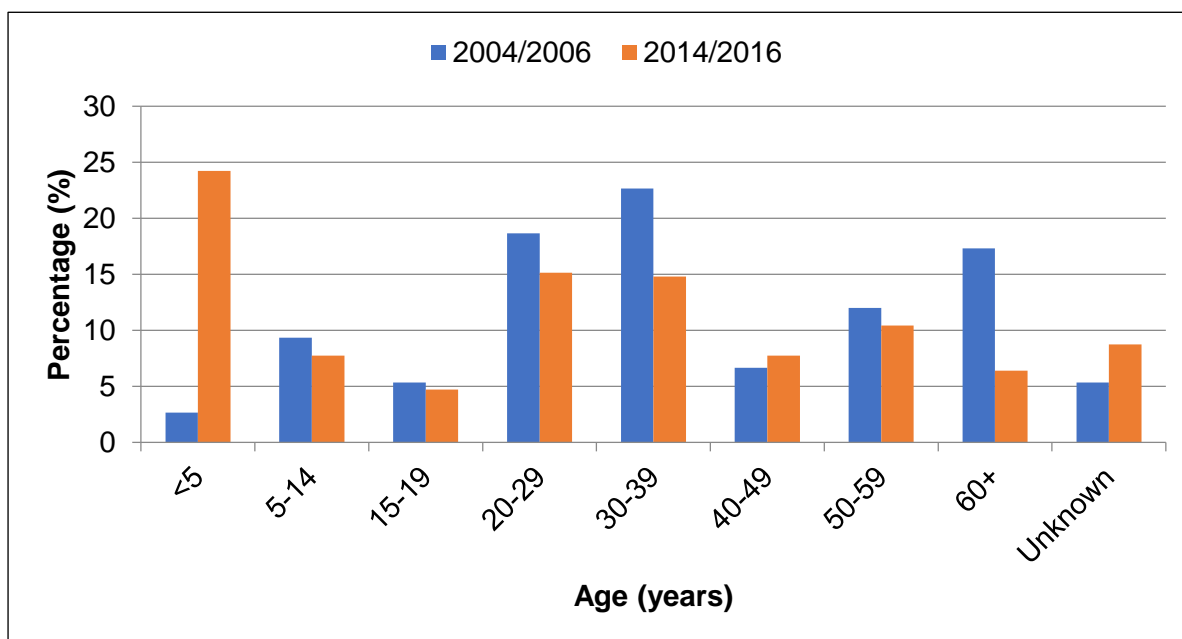
Figure 4.2: Age distribution by study period

## 4.3. Organisms isolated in each of the two study periods

Of the 371 blood culture positive specimens isolated, 204 (55%) were Gram-positive and 55 (14.8%) were Gram-negative microorganisms. The top five most frequent microorganisms derived from the samples included CoNS 190 (51.2%), 44 (11.9%) *Klebsiella* spp, 23 (6.2%) *Enterococcus* spp, and 19 (5.1%) *Enterobacter* spp and 14 (4%) *Acinetobacter* spp. These species accounted for 78.4% of all the pathogens isolated (Figure 4.3).

Figure 4.3: Distribution of microorganisms isolated (2004/06 -2014/16)
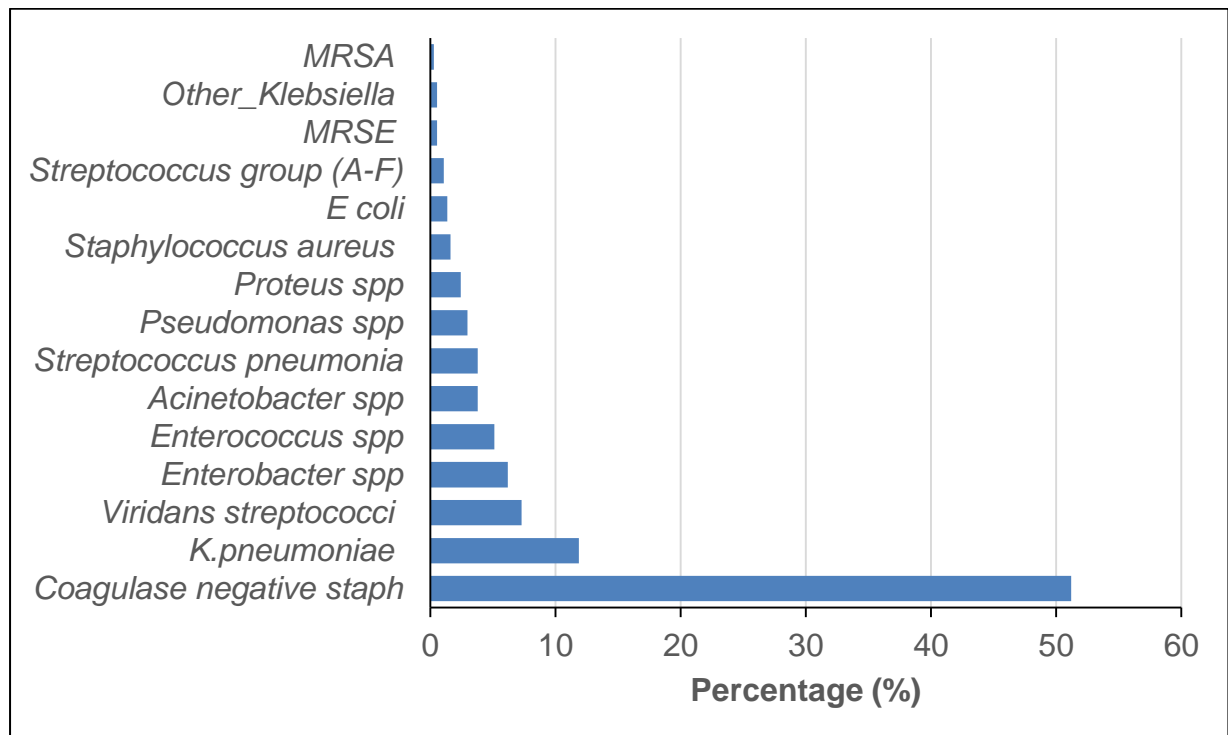


There were no additional/unique organisms in 2004-2006. However, additional microorganisms that were detected in 2014 to 2016 were as follows:

- *Burkholderia cepacia*
- *Candida spp*
- *Citrobacter koserii*
- *Providencia stuatii*
- *Salmonella spp*
- *Stenotrophomonas melophilia*

The CoNS significantly increased from 32% in 2004/06 to 56% in 2014/16, while the *Acinetobacter* spp significantly decreased from 11% to 2% (p<0.05, Table 4.1), respectively. The *E. Coli, Staphylococcus aureus* and *Streptococcus* group *(A-F),* while increasing in numbers, decreased in terms of proportion of isolates (a decrease in proportion p = 0.227, chi square test); while the *Enterococcus* spp and *K. pneumoniae* increased in both numbers and proportions (decrease in proportion p = 0.688, chi square test).

Table 4.1: Pattern of microorganisms per study period

| | 2004-2006 (n=73) | | 2014-2016 (n=298) | | p-values* |
|---|---|---|---|---|---|
| Organism | No | % | No | % | |
| *Acinetobacter* spp | 8 | 11 | 6 | 2 | 0.002 |
| Coagulase negative staph | 23 | 32 | 167 | 56 | <0.001 |
| *E coli* | 4 | 5 | 10 | 3 | 0.489 |
| *Enterobacter* spp | 4 | 5 | 15 | 5 | 0.774 |
| *Enterococcus* spp | 4 | 5 | 19 | 6 | >0.999 |
| *K.pneumoniae* | 8 | 11 | 36 | 12 | >0.999 |
| *Proteus* spp | 2 | 3 | 2 | 1 | 0.175 |
| *Pseudomonas* spp | 2 | 3 | 9 | 3 | >0.999 |
| *Staphylococcus aureus* | 6 | 8 | 21 | 7 | 0.801 |
| *Streptococcus pneumonia* | 2 | 3 | 3 | 1 | 0.256 |
| *Streptococcus* group (A-F) | 3 | 4 | 6 | 2 | 0.388 |
| *Viridans streptococci* | 2 | 3 | 4 | 1 | 0.337 |
| MRSA | 1 | 1 | 0 | 0 | 0.197 |
| MRSE | 2 | 3 | 0 | 0 | 0.038 |
| Other_*Klebsiella* | 2 | 3 | 0 | 0 | 0.038 |

*Fisher's exact test p-values

Table 4.2: Distribution of antimicrobial agents used in susceptibility tests

| | 2004/06 (n=73) | | 2014/2016 (n=298) | | p-values |
|---|---|---|---|---|---|
| | No | % | No | % | |
| Colistin | 3 | 4.1 | 2 | 0.7 | 0.054 |
| Imipenem | 19 | 26.0 | 61 | 20.5 | 0.340 |
| Linezolid | 6 | 8.2 | 187 | 62.8 | <0.001 |
| Meropenem | 19 | 26.0 | 75 | 25.2 | 0.881 |
| Vancomycin | 22 | 30.1 | 215 | 72.1 | <0.001 |

A list of the most commonly used drugs for isolates is shown in Table 4.2. Vancomycin and Linezolid were the main drugs used in the wards for treatment of *S. aureus* infections during the time periods.

Testing for susceptibility against these two antibiotics increased between 2004/2006 and 2014/2016 and this increase was statistically significant.

## 4.5. Drug susceptibility and resistance pattern

Comparison of the susceptibility and resistance pattern of the different microorganisms to various antibiotics per study period is illustrated in Table 4.3.

Coagulase-negative *Staphylococcus* (CoNS) was the most cultured microorganism and showed susceptibility to both linezolid and vancomycin with a significant increase in 2014/16 as compared to 2004/06.

*Staphylococcus aureus* was only observed in linezolid and vancomycin cultures. *Acinetobacter baumanni* was cultured in both colistin and meropenem together with *Klebsiella pneumonia.*

*Escherichia coli* were observed in the carbapenems (imipenem and meropenem).

Table 4.3: Microorganism resistance and susceptibility profiles to antibiotics tested

| | 2004/06 Susceptible | 2004/06 Resistance | 2014/16 Susceptible | 2014/16 Resistance |
|---|---|---|---|---|
| **COLISTIN (n=5)** | | | | |
| *Acinetobacter* spp | | 1 | 1 | |
| *Enterobacter* spp | | 1 | | |
| *K.pneumoniae* | 1 | | | |
| **IMIPENEM (n=80)** | | | | |
| *Acinetobacter* spp | 4 | 1 | 2 | |
| *E coli* | 2 | | 9 | |
| *Enterobacter* spp | 2 | | 11 | |
| *K.pneumoniae* | 7 | 1 | 29 | |
| *Proteus* spp | 2 | | | 1 |
| *Pseudomonas* spp | | | 8 | |
| **LINEZOLID (n=193)** | | | | |
| Coagulase -egative staph | 1 | 1 | 152 | 4 |
| *Enterococcus* spp | 4 | | 12 | |
| *Staphylococcus aureus* | | | 18 | |
| **MEROPENEM (n=94)** | | | | |
| *Acinetobacter* spp | 3 | 2 | 3 | 2 |
| CoNS | | | 1 | |
| *E coli* | 2 | | 10 | |
| *Enterobacter* spp | 2 | | 13 | |
| *K.pneumoniae* | 7 | 1 | 36 | |
| *Proteus* spp | 2 | | 1 | 1 |
| *Pseudomonas* spp | | | 8 | |
| **VANCOMYCIN (n=237)** | | | | |
| CoNS | 13 | | 157 | 5 |
| *Enterococcus* spp | 2 | 2 | 18 | |
| *Staphylococcus aureus* | 1 | | 21 | |
| *Streptococcus pneumonia* | | | 3 | |
| *Streptococcus* group (A-F) | 1 | | 6 | |
| *Viridans streptococci* | | | 3 | |
| MRSA | 1 | | | |

*Numbers differ from those in Tables 4.1 and 4.2 since not all isolates were tested for susceptibility.

Table 4.4: Percentage of resistance pattern to antibiotics

| | Colistin | | Imepenem | | Linezolid | | Meropenem | | Vancomycin | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2004/06 (n=73) | 2014/16 (n=298) | 2004/06 (n=73) | 2014/16 (n=298) | 2004/06 (n=73) | 2014/16 (n=298) | 2004/06 (n=73) | 2014/16 (n=298) | 2004/06 (n=73) | 2014/16 (n=298) |
| *Acinetobacter* spp | 1.4 | | 1.4 | | | | 2.7 | 0.7 | | |
| *Enterobacter* spp | 1.4 | | | | | | | | | |
| *K.pneumoniae* | | | 1.4 | | | | 1.4 | | | |
| *Proteus* spp | | | | 0.3 | | | | 0.3 | | |
| CoNS | | | | | 1.4 | 1.3 | | | | 1.7 |
| *Enterococcus* spp | | | | | | | | | 2.7 | 0.3 |

The resistant pattern of the microorganisms as depicted above shows that *Acinetobacter* spp*.* was resistant to meropenem in both time periods but decreased from 2.7% to 0.7%. The table also showed the resistance pattern to colistin and imipenem in 1.4% specimens in 2004/06. *Enterococcus* showed resistance to vancomycin in both time periods but decreasing from 2.7% to 0.3%. CoNS exhibited resistance to linezolid in both time periods (1.4% to 1.3%) and to vancomycin (1.7% in 2014/16).

With regards to the rate of ESBL and the emergence of CRE amongst the prevalent Enterobacteriaceae, *Klebsiella pneumonia* was found to be predominant, hence the reporting. There was an increase of the ESBL-positive for *K. pneumonia* as indicated in Table 4.1 above in both study periods (2004-2006 and 2014-2016). No CRE were detected in either study period, as shown in Table 4.4 above. Below are the extended spectrum beta lactamases (ESBL) results for those specimens that were evaluated in the two study periods.

Table 4.5 ESBL positivity proportions in the two study periods

| Year | Total | ESBL - | ESBL + | %ESBL + |
|---|---|---|---|---|
| 2004-2006 | 9 | 7 | 2 | 22% |
| 2014-2016 | 34 | 7 | 27 | 79% |

Fisher's exact test p = 0.003
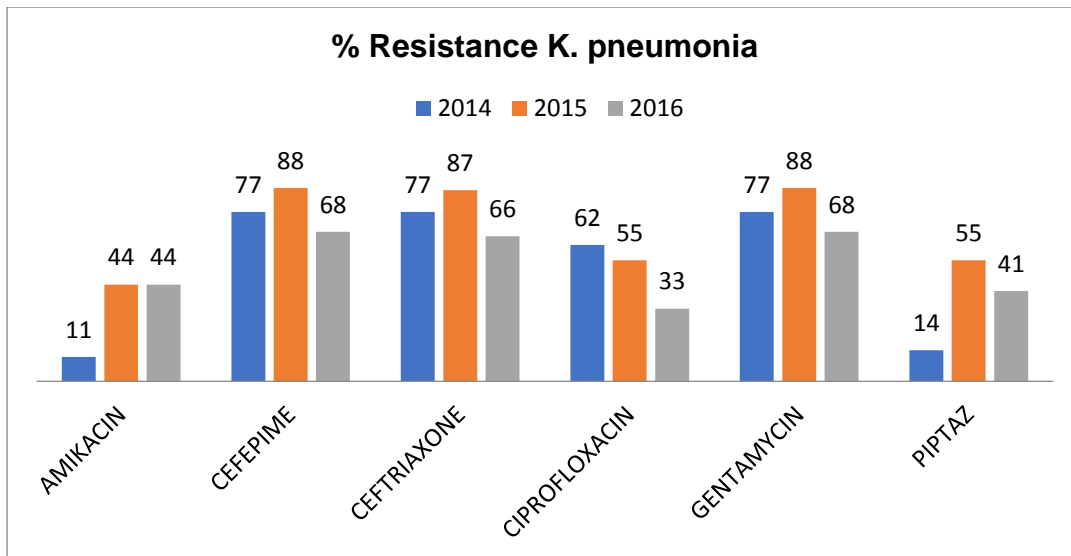
Figure 4.4: Resistance pattern of *Klebsiella pneumonia*

The overall study findings did not show a significant increase in resistance levels over the years as was initially expected.

However, there were variations in resistance amongst the commonly used antibiotics between 2014 and 2016 particularly in *K. pneumonia.* There was an overall increase in resistance percentage from 2014 to 2016 with regards to amikacin, cefepime, ceftriaxone, ciprofloxacin, gentamycin and piptaz. Only ciprofloxacin showed a decline in resistance percentages.

# CHAPTER 5

## DISCUSSION OF THE RESULTS

### 5.1. Introduction

In the previous chapter, Chapter 4, the findings and results of the study were presented.

The results were presented in a form of histograms and tables, and narratives were written about the results depicted in the histograms and tables.

In this chapter, the results are discussed in detail, comparing and contrasting the findings. The hypothesis will also be tested in this chapter.

### 5.2. Discussion

### 5.2.1. Demographic profile of the patients who had bloodstream infections (gender and age)

This study found that, in the period 2004-2006, isolated bloodstream infections (BCIs) were more prevalent in females than in males.

However, in the study period between 2014-2016, more males tested positive for BSIs than females, as indicated in figure 4.1.

A study that was done by Crabtree *et al.*, concluded that the male gender is an independent risk factor for the development of nosocomial BSIs, and this has been associated with in-hospital mortality in septic surgical patients. In the same study, it was found that female gender is an independent predictor of mortality in patients with *Enterococcus* bloodstream infections [53].

A study undertaken in a Norwegian hospital found that the occurrence of significantly more BSIs was related to intravascular devices, endocarditis, skin and wound infections and that there were significantly fewer episodes related to abdominal or genitourinary disease [54].

Female patients in ICU tend to be at an increased risk of developing nosocomial infections. Factors such as indwelling catheters, urinary tract infections expose females to an increased risk of mortality, even after carefully controlling for other prognostic factors [55].

Eachempati *et al.*, demonstrated that critically ill female surgical patients with sepsis may have a slightly increased mortality when compared to their male counterparts [56].

It can be concluded from our study that the increase in male patients can be attributed to the profile of the disease at the time, particularly for the patients admitted in our ICU where most of the BSI were mostly common.

In the period 2004-2006, there were more older patients affected by the BSI than in the subsequent study (2014-2016) as depicted in Figure 4.2.

Studies have demonstrated that older age, chronic renal insufficiency and MRSA were independently associated with mortality within the six month period of acquiring the infection. For every decade increase in age, chronic renal insufficiency was independently associated with in-hospital mortality [57].

Elderly adults show a disproportionate increase in the incidence of sepsis. However, age is an independent predictor of mortality, and younger patients with sepsis have a better chance of survival, while elderly non-survivors of sepsis die earlier during hospitalization, Elderly survivors more frequently require skilled nursing or rehabilitative care after hospitalization [58].

Compared to the younger age group, bacteraemia in the elderly was associated with a different clinical course and a higher mortality rate [58]. As much as the outcome of our study showed that there was an increase in the number of elderly being tested for BSI, the impact of this could not be ascertained.

This study showed that there was an increase in the BSI tests performed on children less than 5 years of age in the study period 2014 to 2016. BSI among children is common, the risk factors associated with these infections are attributed to the process of care rather than to the severity of the illness [59].

BSI among children is mostly due to the insertion of intravascular devices, such as intravenous drips. Children have a low mortality rate due to these infections as the isolates are not different from those in general paediatrics, which are not so virulent. Antibiotic resistance is frequently found in most commonly isolated pathogens, but differences between specialties suggest the existence of local risk factors, some of which might be amenable to infection control interventions [60].

Studies done in the past confirms that hyperglycaemia is common in children and it may be a risk factor for nosocomial infections. Central catheters are also associated with BSIs in critically ill patients [61]. In general, children have significantly higher odds of having a contaminated blood culture than adults which is attributable to the infection control practice [62]. However, our study did not look at the risk factors attributable to the increase in children but an assumption can be made through the acquiring of the pulmonologist who was more vigilant.

Our study demonstrated that more children tested with BSI in the second study period than there were adults who tested with BSI.

### 5.2.2. Types of Microorganisms isolated (CoNS, *Acinetobacter baumanni*, *E. coli*, *Klebsiella pneumonia* and *Staphylococcus aureus*)

### 5.2.3. Coagulase Negative *Staphylococcus* (CoNS)

These microorganisms include all microorganisms which are not *Staphylococcus aureus*. *S. epidermidis* is a major nosocomial pathogen posing significant medical and economic burdens [63] on the health care system. This microorganism was also isolated in most of the blood cultures that were performed in this study, as in Figure 4.3 and Table 4.1 show. Currently the mechanisms as to how the host defends itself against the prototypical CoNS species *S. epidermidis* as a commensal of the skin and mucous membranes is not well understood [63].

Blood cultures which are performed for suspected infection yielded positive results for CoNS.Iit is often difficult to determine whether the presence of this organism in the blood is a pathogen or a contaminant. CoNS isolated in blood cultures are associated with lower levels of inflammation compared to bloodstream infections,

due to known pathogens, and are comparable with patients who have negative cultures [64].

It must be noted that most of the culture isolates were CoNS, whether these were contaminants or true pathogens was difficult to prove as this was not the purpose of the study. CoNS have long been regarded as non-pathogenic. In the recent years their important role as pathogenic microorganisms has been studied. Their specific virulence factors are not as clearly established as their counterpart, *Staphylococcus aureus*, Factors such as bacterial polysaccharide components are involved in attachment and/or persistence of bacteria on foreign materials. Coagulase-negative Staphylococci are by far the most common cause of bacteraemia related to indwelling devices.

Most of these infections are hospital-acquired, and studies conducted over the past several years suggest that they are often caused by strains that are transmitted between hospitalized patients. Common infections caused by CoNS, as mentioned in previous studies, include native or prosthetic valve endocarditis, urinary tract infections and endophthalmitis. Intravenous treatment of systemic infections is usually required because coagulase-negative Staphylococci have become increasingly resistant to multiple antibiotics [65].

This study has demonstrated that CoNS was the most common microorganism isolated in the patients as seen in the bloodstream infections culture results, as indicated in Table 4.1. Most of these patients were those in the ICU and they mostly had indwelling catheters and other intravenous monitoring devices.


### 5.2.4. *Acinetobacter baumannii* (*A. baumannii*)

The other most common and virulent microorganism isolated from the BSI was *A.baumanni* as indicated in Table 4.1. The spread of multidrug-resistant (MDR) *Acinetobacte*r strains among the critically ill, hospitalized patients, and subsequent epidemics, has been an increasing concern from the early seventies [66].

*A.baumannii* strains have been isolated in hospitals and have acquired multiple mechanisms of antimicrobial drug resistance and is a growing public-health problem. Infection control of these outbreaks is difficult to attain due to its complex epidemiology. Implementation of specific control measures, such as identification of

the source of outbreak through surveillance cultures, can assist in combatting the growing concern. Adherence to a series of infection control methods, such as strict environmental cleaning, effective sterilization of reusable medical equipment, attention to proper hand hygiene practices and the use of contact precautions, together with appropriate administrative guidance and support, are required for the containment of these microorganisms [67].

Although the percentage of *A. baumannii* was not so high in this study, owing to its virulent nature, this was a cause for concern, regardless of the number isolated. Furthermore, due to its resistance pattern to antimicrobials, its presence is of utmost importance. *A. baumannii* is a ubiquitous pathogen capable of causing both community and health care-associated infections. It has the propensity to accumulate mechanisms of antimicrobial resistance that lead to pan-drug resistance and can cause large health care-associated infection outbreaks that often involve multiple facilities.

This microorganism mainly causes the following infections: pulmonary infections, urinary tract infections and bloodstream or surgical wound infections.

Invasive procedures, such as the use of mechanical ventilation, central venous or urinary catheters, and broad-spectrum antimicrobials have been identified as the major risk factors in acquiring the infection from the bacteria [68].

*A. baumannii* has the ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces, This ability has made this organism a frequent cause of outbreaks of infection and an endemic, health care-associated pathogen [69]. Multidrug-resistant *Acinetobacter* infection usually occurs in severely ill patients admitted to the ICU. The associated crude mortality rate tends to be high, ranging from 26% to 68% [69]. However, in our study no mortality rate was reported as that was not the purpose of the investigation [70].

**Exacerbating factors**

Antimicrobial exposure

Mobile resistance elements

Antimicrobial-susceptible

**Exacerbating factors**

| | |
|---|---|
| Prolonged hospital stays | Exposure to ICU |
| Mechanical ventilation | Severity of illness |
| Recent surgery lapses | Infection control |

**Mitigating factors**

Antimicrobial stewardship

Antimicrobial-resistant

Acinetobacter

**MDR-Acinetobacter**

**Colonization and infection**

**Mitigating factors**

| | |
|---|---|
| Hand hygiene | Cleaning and disinfection of equipment and the environment |
| Standard precautions | |
| Appropriate isolation precautions | Cohorting of staff and patients |
| | Surveillance |

Community hospitals

Health care associated transmission
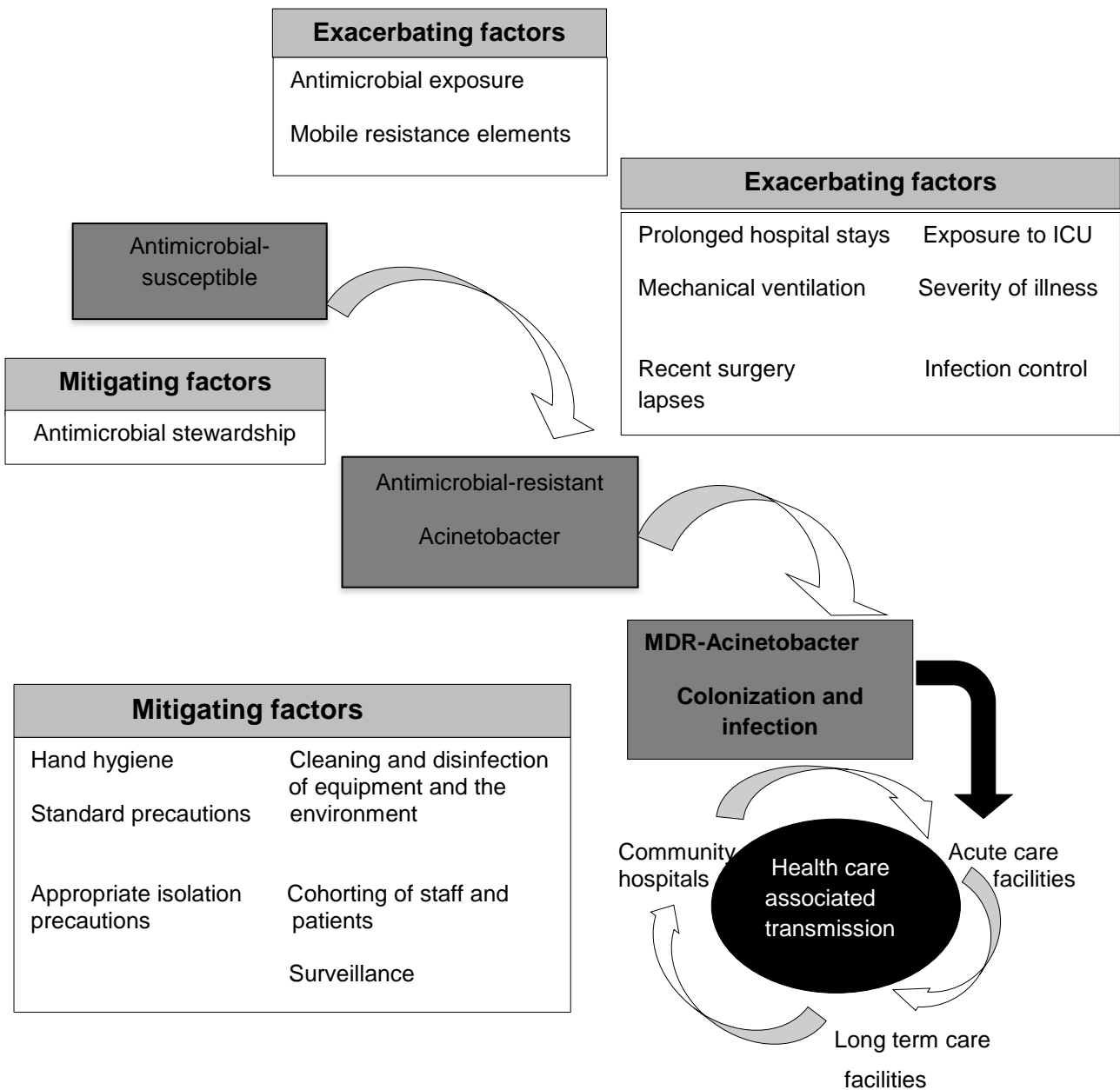
Acute care facilities

Long term care facilities

Figure 5.1: Factors leading to the emergence and transmission of multi-drug-resistant (MDR) Acinetobacter species in intensive care unit [69].

### 5.2.5. *Klebsiella pneumonia* (*K. pneumonia*)

*K. pneumonia* was one of the top five micro-organisms isolated in the BSI isolates studied. Studies conducted in the past found that community-acquired pneumonia was only found in Taiwan and South Africa. Invasive syndrome of liver abscess and meningitis, or endophthalmitis occurred only in those countries based on the study at the time. Community-acquired bacteraemia was defined as a positive blood culture taken on admission or within 48 hours of admission. Site of infection accompanying the bacteraemia was determined as pneumonia, urinary tract infection, meningitis, incisional wound infection, other soft tissue infection, intra-abdominal infection, and primary bloodstream infection [71].

However, in this study *K. pneumonia* was only isolated in admitted patients. *K. pneumoniae* has been a recognized pulmonary pathogen since its discovery greater than 100 years ago. *K. pneumoniae* continues to be associated with community-acquired pneumonia in Africa and Asia [72]. However, this study could not establish whether the isolated *K. pneumonia* was also based on the assumption above but that it was hospital acquired. The findings in this study only showed an increase of 11% to 12% in both time periods of the *Klebsiella* spp isolated; however, it was not statistically significance.

### 5.2.6. *Escherichia coli* (*E. coli*)

Extended-spectrum-β-lactamase (ESBL)-producing strains of *E. coli* are common in hospitalized and non-hospitalized patients, they are responsible for BSIs [73]. Bacteraemia is the leading cause of morbidity and mortality mostly associated with Gram-negative rods (especially *E. coli*). *E. coli* is also mostly associated with community acquired BSI [74]. However, this study was conducted in the hospital setup and an assumption can be made that those isolated were nosocomial. It must be noted that the epidemiology, reservoir and the principal routes by which humans are exposed are both still poorly understood [74].

*E. coli* is among the best-known bacterial species and one of the most frequently isolated organisms out of the clinical specimens in the bloodstream [75].

However, this study, indicated that despite being isolated, *E, coli* was not among the highest microorganism detected and it was not clinically significant.

Data obtained from NHLS was poorly captured as to indicate the time when patients were admitted in the ICU. In addition, the admission book in ICU was poorly recorded where a lot of data for the time could not be established. The date of admission and the date of diagnosis after 48 hours could not be established.

A study conducted by Weinstein et al., found that the distribution of Gram-negative pathogens associated with BSIs in ICUs changed very little and *E. coli* was somewhat less frequently reported. These authors also reported that the change was not statistically significant [76].

This finding concurs with the finding of this study, which was alluded to in our results as per the previous chapter.


### 5.2.7. *Staphylococcus aureus* (*S. aureus*)

*S. aureus* is one of the micro-organisms that was isolated in bloodstream cultures that were performed in this study. In a study done in Nigeria, *S. aureus* emerged as a major public health concern because of the multi-drug resistant strains that were found.

The same study found \ a high percentage of the vancomycin resistant *S. aureus,* which could have resulted from compromising treatment options and inadequate antimicrobial therapy [77]. Outbreaks of hospital-acquired infections caused by methicillin-resistant *S. aureus* have been found to be increasing in frequency in the United States. Most of these outbreaks were cantered in intensive care units, which posed a big challenge. Patients who were infected and hospitalized were found to be microbe reservoirs and the health care worker's hands acted as transient carriers from patient-to-patient. Methicillin-resistant strains of *S. aureus* (MRSA) have become established endemic nosocomial pathogens [78]. *S. aureus* infection showed a significant increase in the past decade and it was associated with very high mortality rates of between 15% and 60%. *S.aureus* isolates which are resistant to methicillin has become a growing challenge and most of the nosocomial infections in ICUs are due to methicillin-resistant *S. aureus* [79].

The *Staphylococcus* isolated in the BSIs in this study were all nosocomial, which posed the same challenges as alluded to with respect to other studies as per the literature.

Even though MRSA is an increasingly common pathogen, assumptions about a patient's outcome cannot only be attributable to methicillin resistance because most patients who develop MRSA are older and sicker than those who develop MSSA infections [80].

While MRSA studies have been undertaken worldwide, there is paucity of data available from, South Africa as shown in a study done in Kwazulu Natal [81]. This is in line with the findings of this study, where there were very few isolates. However, the very nature of MRSA poses a serious problem. *S. aureus* remains a versatile and dangerous pathogen in humans*. S. aureus* infections, both community acquired and nosocomial infections, increased steadily with very little change in the mortality [82]. A study conducted in Cape Town, South Africa found MRSA to be the predominant nosocomial pathogen in children. However, *S. aureus* bacteraemia remained stable in children over the five years studied [83].

In this study the *S. aureus* isolated was not specific to children but also never showed any changes.


### 5.2.8. Drug resistance and sensitivity pattern (vancomycin, linezolid, carbapenems (imipenem and meropenem) and colistin

### 5.2.8.1. Vancomycin resistance and sensitivity pattern

Vancomycin resistance and sensitivity pattern as described in a previous chapter of this study, was observed mostly in the coagulase negative *Staphylococcus aureus*. Only two isolates showed resistance out of those that were studied.

Studies done in the United States found that VRE demonstrated that patient-to-patient transmission of the microorganisms occurred either via direct contact, indirectly via the hands of personnel, via contaminated patient-care equipment or via environmental surfaces [84]. Since this study was a descriptive study, it was not easy to evaluate whether this finding was in line with the findings of this study. However, an assumption can be made that the resistance observed can be attributable to the findings from other studies.

Subsequent isolation of several vancomycin-resistat *S. aureus* (VRSA) strains have been observed in the USA, France, Korea, South Africa and Brazil and VRSA is becoming a global challenge [85].

In this study, resistance to vancomycin was observed in the enterococcus species identified from the analysed results during both the study periods.

The emergence and dissemination of high-level resistance to vancomycin in enterococci can lead to clinical isolates resistant to all antibiotics. Enterococci microorganisms are not that highly pathogenic but clinicians must be vigilant about the emergence of these isolates as they could cause challenges in the future [86].

CoNS are commonly resistant to antibiotics that are indicated for staphylococcal infections, with the exception of vancomycin [87]. However, this study indicated that resistance to two isolates was observed.

A study undertaken in Brazil confirmed that coagulase-negative Staphylococci clinical isolates were heteroresistant to glycopeptides (e.g. vancomycin). The same study concluded that the detection of heteroresistant organisms justifies the judicious use of vancomycin and teicoplanin [88].

### 5.2.8.2. Linezolid resistance and sensitivity pattern

Results in the previous chapter indicated that most of the microorganisms isolated were sensitive to linezolid. However, a resistance pattern was also observed in CoNS. The new oxazolidinone antimicrobial, linezolid, has been approved for the treatment of infections caused by various Gram-positive bacteria, including MRSA and vancomycin-resistant enterococci (VRE) [89].

The antibiotic was used for its intended purpose, which is the treatment of infections caused by Gram-positive microorganisms.

A study conducted in Brazil found that if linezolid is not used in optimum dosages there is a high risk of developing resistance. The conclusion was that, in order to prevent this untoward effect, optimal dosage of linezolid must be used to prevent the emergence of resistance [90].

Epidemiological studies done in the past indicated that linezolid resistannce to *S. aureus* isolates is low. Linezolid-resistant *S. aureus* emerged due to prolonged therapy [91]. This study did not have any isolates which indicated *S. aureus* resistance. Surveillance studies done in the past have indicated that only less than 0.1% of CoNS are linezolid-resistant. Linezolid-resistant CoNS can be attributed to person-to-person spread, which leads to the establishment of skin colonization [92].

Linezolid resistance is uncommon among Staphylococci, but approximately 2% of clinical isolates of CoNS may exhibit resistance to linezolid [93].

However, this study has shown that 1.4% and 1.3% of the isolates in 2004/06 and 2014/16 respectively for CoNS were resistant and that this can also be attributable to person-to-person spread owing to the poor practice of infection control measures. *S. epidermidis* could emerge as an outbreak in the intensive care units and, as such, resistance strains could develop due to increase usage of linezolid, will subsequently give rise to increased resistance. Therefore, restrictions on linezolid usage and infection control measures must be introduced to control the outbreak [94].


### 5.2.8.3. Carbapenems resistance and sensitivity pattern

The two antimicrobials which constitute the carbapenems are meropenem and imipenem. The previous chapter indicated that the two were isolated in the bloodstreams cultures isolated from the study periods. Gram-negative bacilli usually form part of the intestinal flora, which is their major reservoir. These microorganisms include *Enterobacteriaceae* and non-fermenters such as *Pseudomonas aeruginosa (P.aeruginosa)* and *A. baumannii*, all of which are potentially pathogenic for patients hospitalized in intensive care units. Carbapenems are currently the only active beta-lactams effective against the above-mentioned microorganisms, which has led to an increase in their use, not only for documented infections, but also for empirical treatment of acquired hospital infections such as those occurring in ICU patients [95].


**Imipenem:** Imipenem-resistant gram-negative bacilli are usually associated with more severe clinical outcomes resulting in higher morbidity and mortality, especially when the infection is acquired in ICU [95].

This study found that there were only 3 isolates of *Acinetobacter, K. pneumonia,* both at only 1.4%; and *Proteus species* at 0.3% where imipenem resistant was observed. A study conducted in Iran found that 55% of *A. baumannii* isolates were resistant to imipenem. Of those, 74% had a multidrug resistance phenotype. In the same study they found that, although high, this level of multidrug resistance was still low compared to countries such as Kuwait [96]. This study demonstrated a low percentage, as described above, but that this was a cause for concerned as one resistance can render the antibiotic useless for the use in the isolated microorganism. CoNS is the most common microorganism isolated from BSIs. Studies conducted in the past have shown that the empirical use of imipenem will soon render most of the organism resistant to this antibiotic [97]. Care should be taken in the use of this antimicrobial, especially its empirical use without blood cultures, which is the case in the Pietersburg Provincial Hospital. Prior use of carbapenems is strongly associated with *A. baumannii* resistance, therefore this practice must be avoided [98]. Carbapenem-resistant *K pneumoniae* isolates are rapidly emerging These isolates are usually resistant to virtually all commonly used antibiotics and there is a need for strengthen control of their spread because, without control, it will pose a serious challenge [99]. This study showed resistance to *K. pneumonia* but the percentage was low, which indicates an emerging threat of resistance to this antimicrobial.

**Meropenem**: Meropenem is a parenteral carbapenem antibiotic with excellent bactericidal activity *in vitro* against almost all clinically significant aerobes and anaerobes. It has an antibacterial spectrum broadly similar to that of imipenem. However, it is slightly less active against staphylococci and enterococci [100].

*A. baumannii* was isolated in 2 isolates in both time periods and they were found to be resistant to meropenem. The increasing trend of carbapenem resistance in *A. baumannii* worldwide is a cause for concern, since it drastically limits the range of alternative therapeutic agents. *A. baumannii* is an opportunistic pathogen frequently involved in outbreaks of infection, occurring mostly in intensive care units [101].

This microorganism is now being isolated more frequently, particularly in intensive care settings. It causes serious infections, such as ventilator-associated pneumonia, bloodstream infection, urinary tract infection, meningitis and wound infection. Mostly it affects severely immunocompromised patients, predominantly found in ICUs. *A. baumannii* is found rarely on human skin, it is not a normal environmental organism and its natural reservoir is relatively unknown [102].

The fact that two of our isolates exhibited resistance to meropenem for this microorganism is a very serious cause for concern and drastic measures need to be instituted.

A study done in a Johannesburg hospital in South Africa found that development of resistance was due to response to antibiotic pressure. The spread of resistant strains was as a result of health care worker and/or patient transfer among hospitals. The study emphasized the need to institute stricter infection control measures to limit the spread of *Acinetobacter* among hospitals [103]. This finding was in line with findings of this study with respect to one of the patients, who was a referral from a neighbouring hospital. However, this did not form part of this study. The other resistance strain isolated in the BSI in this study was identified as *K. pneumonia*. A study done in South Africa found that *K. pneumoniae* was cultured from 41.2% complicated intra-abdominal infections in private hospitals and in 55-74% bacteraemic isolates in the public sector. The same study found that CRE have indeed become the 'worst nightmare', locally and internationally, and posed a major threat to the viability of all currently available antibiotics [104].

Carbapenems (imipenem, meropenem, and ertapenem) studies done in the past revealed that there is insufficiency in treating enterobacterial infections with *K. pneumonia* carbapenemases-producing bacteria, which are, in addition, resistant to many other non-β-lactam molecules, leaving few available therapeutic options [105]. However, this study only depicted one isolate, which showed resistance to meropenem in the two study periods.

The emergence of resistance to meropenem in *K. pneumoniae* may be attributable to the prolonged treatment with the antimicrobial (meropenem) and in the absence of apparent foci of infection as is the case with empirical treatment [106].

### 5.2.8.4. Colistin resistance and sensitivity pattern

This study demonstrated that colistin resistance was found in two isolates (*A. baumannii* and *Enterobacter* species). Treatment with colistin is the last resort for management of multidrug-resistant *A. baumannii*. Reports for colistin resistance have begun emerging throughout the world.

The highest resistance rate was reported in Asia, followed by Europe. Pharmacokinetic/pharmacodynamic studies have revealed that colistin monotherapy is unable to prevent resistance, and combination therapy might be the best antimicrobial strategy against colistin-resistant *A. baumannii* [107].

A study conducted in Ireland showed that the emergence of colistin resistance amongst *Enterobacteriaceae i*solates, mostly those of *Enterobacter* spp, requires that laboratories monitor these trends, because of growing antimicrobial resistance and therapeutic options are diminishing [108].

In the present study, as the percentage of the resistance for Enterobacter was low, it warrants the same vigilance as recommended by the study conducted in Ireland because we could run out of options in the future. A study done in Hungary showed that colistin resistance was 0.6% for *Enterobacteriaceae* and 2.6% *Acinetobacter* spp*.* The same study found that colistin-resistant strains were in accordance with other findings in other European studies.

While the prevalence of resistance was low, the heteroresistance was significantly higher, however, the clinical significance phenomenon was unclear [109]. In this study the resistance to both *Enterobacteriaceae* and that of *A.baumannii* was 1.4% for both, which was also low. There is a critical need for effective prevention of infection, control measures and strict use of antibiotics, globally, is required in order to control the rise and spread of resistance to colistin [110].

**5.2.3.5 Extended spectrum beta lactamases (ESBL) positive isolates**

"ESBLS are Gram-negative bacteria that produce an enzyme; beta-lactamase that has the ability to break down commonly used antibiotics, such as penicillins and cephalosporins and render them ineffective for treatment".

The most common ESBL-producing bacteria are some strains of *E. coli* and *K. peumoniae* [111]. This study found that the ESBL positive isolates in the specimens tested were very few. Prevalence of resistance statistics from many parts of the world are unavailable. However, accumulating evidence suggests that resistance to extended-spectrum cephalosporins in *E. coli* and, in particular, *K. pneumoniae* has become a worldwide problem [112]. In this study, even though the numbers are few, the impact may outweigh the concerns of the few isolates observed.

**5.3.   Antibiotic stewardship in Pietersburg hospital**

A committee for the antibiotic stewardship has recently been formed at the Pietersburg Provincial Hospital in order to address the rational use of antimicrobials. This committee will assist in addressing the formulation of policy for antibiotic use, which at the time of the study was non-existent. It must also be noted that this committee should assist in monitoring the levels of resistance, guarding against the raise in resistance patterns in the institution. This requirement is as per guidance from the South African National Department of Health. The committee should also assist in monitoring compliance and adherence in order to ensure quality.

**5.4.   Laboratory methods and susceptibility testing**

Human morbidity and mortality in critically ill patients is often caused by BSIs. Patients suspected of having a BSI are routinely evaluated using blood cultures, which optimally yield an aetiological diagnosis. Antimicrobial susceptibility testing usually provides a guide for therapeutic intervention, when necessary. Traditional principles of the selection of patient suspected of having BSI, adequate and careful specimen collection observing the aseptic techniques, appropriate cultivation and accurate result interpretation by an experienced clinical microbiologist remain critical

to the delivery of the most effective care for the patients suspected of having BSIs [113].

In the event that BSI is suspected, blood cultures remain the most common specimens sent to the microbiology laboratory.

## 5.5.    Observed patterns during the two study periods

There were organisms which were not observed in the first study period, which ten years later were observed. This observation was in line with the points raised in 5.4. It can be assumed that there was an increase in the numbers of resistant organisms isolated.

Sixty-five years ago, before patients were treated with antimicrobial agents, bacteria isolated from these patients had almost no resistance genes. However, after each new antimicrobial became widely used, a gene expressing resistance to it ultimately emerged.

Over the years, when antimicrobial agents became widely used, eventually a resistant strain emerged [114]. The increase in the extensive use of antibiotics in the community and hospitals has fuelled the crisis of the emergence of resistance strain as the years went by [115]. However, in this study, there were no clinically significant changes in resistance proportions and resistance levels remain below 3%. Sensitivity of the testing methods could have influence the low resistance that was identified.

# CHAPTER 6

## CONCLUSIONS AND IMPLICATIONS

### 6.1. Introduction

This chapter aims to draw conclusions from the results in Chapter 4 and the discussion in Chapter 5. Conclusions will be aimed at the research question and the hypothesis drawn from the beginning of the study.

The limitations of the study as well as the need for future research in order to close the gaps identified will be highlighted in this chapter.

### 6.2. Conclusion about each research question or hypothesis

The research question was asked and the conclusion drawn after the study is as follows:

At the beginning of the research, or the initiation thereof, the assumption was that BSIs were common in hospitalized patients. Inadequate treatment resulted in mortality and an increase in the number of resistant organisms.

Surveillance studies provided important information that allowed identification of trends in pathogen incidence and antimicrobial resistance. This study was initiated because there was an assumption that there was limited published data on the prevalence and antimicrobial profile of bacterial pathogens isolated from the BSIs of patients admitted to the Pietersburg Provincial Hospital.

The study periods that the results were observed from indeed yielded different results.

### 6.2.1. The study hypothesis

The hypothesis tested was that there is a change in the levels of antibiotic resistance among infectious agents isolated from the Pietersburg Provincial Hospital's ICU and that this differed between the two study periods.

The null hypothesis stated that there was no change. It must be noted that the BSIs observed were only from patients admitted to ICU.

Resistance levels remained below 3% and no clinically significant changes were detected in this study.

### 6.2.2. Study objectives conclusions

- Organisms isolated in the Pietersburg Provincial Hospital's ICU in each of the two comparisons time periods were described as per this objective. It was concluded that there was indeed a difference as per the results observed.
- There was an increase in the number of CoNS isolates and a decrease in *Acinetobacter* spp.
- There was an increase in testing against linezolid and vancomycin.
- Drug resistance levels did not change much over the study period and specifically there was no change in the CRE.
- ESBL may have been on the increase but due to small and possibly biased numbers/samples this cannot be certain; vigilance is required.

### 6.3.  Conclusion about the research problem

The following conclusions were drawn from the study:
- There was an increase in the number of resistant microorganisms over the previous ten years.
- There was an increase in the number of male patients, compared to female patients, who were admitted to the hospital with bloodstream infections.
- Coagulase-negative Staphylococcus was the most documented microorganism in the bloodstream infections isolated over the years.
- Despite the increase, most of the microorganism isolated is still sensitive to the antimicrobials.

**6.4 Implications, contribution and application of the study in clinical care at Pietersburg Provincial hospital**

**Implications**

This was the first study done in this hospital and will serve as a baseline data to refer to in the future. The findings revealed a need to provide adequate information when requesting laboratory investigations for effective utilization of the laboratory. The high number of skin commensals that were isolated indicates that blood culture collection technique need to be reviewed.

The study will assist clinicians to formulate the guidelines and protocols for the rational use of antibiotics which at the time of study were not available. The study will also assist clinicians to start empirical antimicrobial treatment where necessary while awaiting blood culture results.

**Contribution**

This Study will contribute in the antimicrobial stewardship programs of Pietersburg Provincial hospital which seek to optimize antimicrobial prescribing in order to improve individual patient care as well as reduce hospital costs and slow the spread of antimicrobial resistance.

The study findings will also serve as an educational tool for clinicians to reflect on their tendencies with regards to the detected high contamination rate of blood cultures, review their practices and develop protocol that will enhance appropriate utilization of the laboratory services.

**Application**

With antimicrobial resistance on the rise worldwide and few new agents in development, it rests with Pietersburg Provincial hospital to curb the irrational prescribing of antimicrobials in ensuring the continued efficacy of available antimicrobials.

The design of antimicrobial management programs should be based on the best current understanding of the relationship between antimicrobial use and resistance.

Pietersburg Provincial hospital through the help of this study will make sure that multidisciplinary teams which are composed of infectious disease, physicians, clinical pharmacists, clinical microbiologists and infection control practitioners are available and should be actively supported by hospital administrators.

**Recommendations:**

- The antimicrobials prescription protocols and stewardship programme should be established, including training of all hospital workers.
- Infection control measures must be reviewed or maintained and monitored in the hospital.
- Medical doctors should be trained about evidence based and rational use of antimicrobials.

## 6.4.    Limitations

The fact that the data were routinely collected and reviewed retrospectively meant that there were missing data regarding the nature of the illness and the health outcomes. This limited the extent of the analysis possible.

The fact that there was no full-time doctor in the intensive care unit results in fewer blood cultures being performed. There may, therefore, be bias in terms of the patients selected for blood cultures.

In the laboratory, further processing involved the removal of the positive blood culture bottle from the machine, followed by culturing on respective agar plates to recover isolated colonies of organisms for identification and susceptibility testing. The two processes were done manually or by the use of semi-automated instruments from various manufactures. This background is provided to explain the circumstances and the huge variation of the findings in the two-study periods.

The following limitations were also noted:

- Data for 2004- 2006 was very scant, possibly due to the change in the laboratory information system and the lack of input from the microbiologist at that time.

- Capturing of data was predominantly manual and could not be retrieved with the current system.

- The microbiology testing was not centralized in the first-time period; – inconsistent reporting standards and testing protocols may have been an issue during this time.

- No input from on-site pathologist, especially with the drug to organism matches, when reporting.

- Lack of specialist or senior doctors in the ICU – the hospital was primarily a regional hospital.

- The susceptibility testing was done manually using the disc diffusion method.

- The reports were manually entered and reports filed. There was no electronic capturing and storage for the reports.

The researcher assumed that it was for the reasons mentioned above, that very limited data were available in 2004-2006. However, it could also be due to the fact that the hospital was primarily a regional hospital with no laboratory specialists.

Data for 2014-2016 had increased due to the following:

It was assumed that the improved yield or turnaround time increased the utilization of the laboratory. Other additional factors considered were the fact that the institution is currently a provincial tertiary hospital admitting referrals which would need investigations, including blood cultures.

From the laboratory point of view, the availability of an onsite microbiologist in 2012 could have added value to clinicians in understanding the role of blood cultures in patient care.

## 6.5. Further research

It is recommended that prospective studies should be carried out in all hospitals in the province to monitor adherence to good antibiotic stewardship. These studies may be coupled with periodic drug susceptibility surveys to identify problem areas.

There is a need to compare the overall admissions to the number of patients developing nosocomial BSIs in order to arrive at the proper proportions.

The contributing factors leading to the increase in nosocomial bloodstream infections in Limpopo should be determined.

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