Methicillin-Resistant Staphylococcus Aureus bacteraemia in adults at Chris Hani Baragwanath Academic Hospital.

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

Johannesburg, 2019

Dedication

I would like to show my appreciation to my remarkable wife, Lara, for all her love, assistance and complete understanding. She has been by my side during my postgraduate studies through all the late nights and countless hours of encouragement.

Presentations originating from this research

Poster presentation at the 34th World Congress of Internal Medicine, Cape Town, South Africa 18 – 21 October, 2018.

Acknowledgements

I would like to show my appreciation to Prof Colin Nigel Menezes and Dr Faieza Sahid for assisting and guiding me with my protocol, data collection and writing of this research report. Their guidance as supervisors has made the path of doing this research project considerably smoother. I would also like to express thanks to Mr Senyo for assisting me with the statistics.

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Abstract

Introduction: The advent of methicillin-resistant Staphylococcus aureus (MRSA) has led to poor patient outcomes and longer hospital stays. Initially described as a nosocomial infection, it has now led to the development of community-acquired (CA) strains. This study aimed to describe the differentiation between CA and hospital-acquired (HA) MRSA infections.

Methods: A retrospective study was conducted at Chris Hani Baragwanath Academic Hospital in Johannesburg from 1 January 2013 to 31 December 2015. Cases were detected utilising the National Health Laboratory Service. One hundred adult patients that met the inclusion criteria were included in the study. Descriptive statistics was used using the Pearson chi-squared test and a p value of less 0.05 with a confidence interval of 95% was used as statistically significant.

Results: Seventy-seven cases were HA, 5 cases were CA and 18 cases were healthcare-associated (HCA). The all-cause mortality was not statistically different between the three groups, but the all-cause mortality was higher in HIV positive patients (61% vs 38%, p value <0.05). Forty-two of 43 cases in the surgical department were HA (97.7% vs. 2.3%); 4 of 5 cases of CA-MRSA and all 18 cases of HCA-MRSA were medical patients. Thirty-nine patients (39 %) were admitted to the intensive care unit. Fifty-seven patients (57%) were HIV-positive, and 34 (60%) were on antiretroviral therapy. Soft-tissue (24%) and catheter-associated infections (21%) were the two most common sources of infection in HA-MRSA patients. HA-MRSA had a lower Charlson co-morbidity index compared to both HCA-MRSA and CA-MRSA (3 vs 6 and 6 respectively, p< 0.05).

Conclusion: Rates of CA-MRSA infections remain low in our setting compared to international data, but may be underestimated due to the small sample size. While HA-MRSA is common in surgical patients, HCA-MRSA makes up the majority of cases in medical patients, with co-morbidities and previous hospital exposure important determinants.

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Nomenclature

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CA	Community acquired
$CD4_+$	Cluster of differentiation 4
CDW	Corporate data warehouse
CHBAH	Chris Hani Baragwanath Academic Hospital
CRP	C reactive protein
HA	Hospital acquired
HCA	Health-care associated
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IQR	Interquartile range
LA	Livestock associated
mecA	Methicillin resistance determined
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NHLS	National Health Laboratory Service
PBP2A	Penicillin-binding protein
SAB	*Staphylococcus aureus* bacteraemia
SCCmec	*Staphylococcal* cassette chromosome methicillin-resistance determined
SOFA	Sepsis-related organ failure assessment
UK	United Kingdom

USA	United Stated of America

Chapter 1: Protocol with extended review of the literature

1.1 Introduction

Staphylococcus aureus (*S. aureus)* is a coagulase-positive gram-positive coccus of the family Micrococcaceae. It is a human commensal in up to 25 – 30% of healthy people, the most common site of colonization is the anterior nares followed by skin, oropharynx, vagina, axilla and perineum [1].

S. aureus remains one of the most virulent organisms with the ability to cause a wide spectrum of diseases, varying from minor skin infections to life-threatening infections. Intrinsic virulence along with host-related factors take part in the development of clinical disease [1,2]. Bacteraemia due to *S. aureus* remains an important cause of morbidity and mortality, but with the emergence of Methicillin-resistant *S. aureus* (MRSA) it has resulted in poor patient outcomes, longer hospital stays and an increased financial burden [3]. Mortality related to *S. aureus* infection drastically decreased with the discovery of penicillin, but in less than two decades after the development of the first cases of penicillin resistance, 80% of all infections caused by *S. aureus* were penicillin resistance. Following the advent of a semi-synthetic penicillin (methicillin) a new species namey MRSA emerged. The initial cases of MRSA were initially only attributed to nosocomial infections, but have now spread into the community resulting in infections in patients who have recent as well as no prior hospital exposure [4]. The development of resistance is somewhat attributed to the inappropriate and indiscriminate use of antimicrobials – not only by the healthcare sector, but also the agricultural sector – and the increase in globalization with the resultant increase in international travel has resulted in swift spread across international borders [4].

MRSA bacteraemia make up 30% of *S. aureus* bacteraemia (SAB) with 7% being community-acquired MRSA (CA-MRSA).The data on MRSA bacteraemia from one of the largest hospitals in the world Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg is limited [5,6]. It is therefore important to study this bacterium in our setting to ensure that adequate preventative and treatment modalities can be implemented based on the epidemiological features of *S. aureu*s.

1.2 A brief review of the history, microbiology and resistance of MRSA bacteraemia infections

The development of antibiotic resistance, especially that of *S. aureus*, has been of great concern. This is related to its inherent virulence, spectrum of clinical disease and its ability to adapt to its environment [4]. MRSA resistance occurs due to a large mobile genetic element known as staphylococcal cassette chromosome mec (SCCmec, methicillin resistance determined) that gets introduced into the methicillin-sensitive strain, this results in the production of a penicillin-binding protein (PBP2A) that reduces the affinity for ß-lactam antibiotics. Five different SCCmec's with various subtypes has been described [1].

The management of *S. aureus* infections using penicillin and its derivatives were initially very effective. However, soon after the introduction of penicillin the preliminary resistance reports reported resistance patterns of up to 50%. There was a growing public health concern with the emergence of a highly invasive penicillin-resistant strain; this ST-80/81 strain was first observed in Australia in 1957, with the advent of methicillin this strain soon disappeared, but resistance to methicillin emerged six months after the drug came onto the market in the1960s [1,7].

In Australia, CA-MRSA initially appeared in the early 1990s with risk factors attributed to low-income communities, aboriginal communities, school children, incarceration, soldiers, athletes and homosexual men [8]. Up to the 1990s MRSA infections were restricted to the hospital setting and was usually limited to a special patient population: the young, elderly, immunocompromised or surgical patients [7]. The first well-documented cases of CA-MRSA were isolated in children in the United States of America (USA) in the late 1990s. These initial cases were primarily in children that had severe sepsis and carried a high mortality rate [7].

With regard to antibiotic resistance, CA-MRSA can be told apart from hospital acquired (HA)-MRSA in three ways. Firstly CA-MRSA harbours different SCCmec's (IV and V), is usually less resistant to other antibiotics than methicillin and beta-lactams (with the exception of USA 300 strain) and lastly also usually have lower minimum inhibitory concentrations (MIC) than HA-MRSA [7]. Genetically different strains of CA-MRSA are growing in number, with more than 20 different genetic lineages. Worldwide there are five dominant strains, namely ST1-IV (WA-1, USA400), ST8-IV (USA300), ST30-IV (South West Pacific clone), ST59-V (Taiwan clone), and ST80-IV (European clone). Mathematical models have predicted that in future, cases of CA-MRSA will replace those of HA-MRSA [9].

1.3 Epidemiology of MRSA bacteraemia infections

S. aureus remains a common infection not only for hospitalized patients, but also within the community [10]. MRSA resistance started in the early 1950s, however, by the early 2000s the resistance patterns in South-Africa were not as well established [10].

Europe:

In Europe considerable interest into MRSA started during the early 1960's. The rates of MRSA in Denmark where as high as 40%, this prompted the Danish to start a large population based study where they described the initial increase in SAB from 3 to 20 per 100 000 members of the population and by the end of the study period they have successfully reduced the rates of MRSA to 0.1% [11]. The rate of MRSA in other countries in Europe was established at 20% with certain countries including the UK, Belgium, Ireland, Germany and the Netherlands showing a noteworthy increase. A study from the UK showed an increase in the number of cases of MRSA from 1997 – 2004 with 24% of cases being CA-MRSA. Other regions had similar rates of MRSA between 10 – 24% these included France, Hungary, Poland, Czech Republic, Latvia and Switzerland. The lowest prevalence of MRSA Bacteraemia was reported in Estonia, Denmark, the Netherlands, Iceland, Finland and Sweden where the proportion of MRSA bacteraemia was less than 5%. The incidence of MRSA Bacteraemia in a study done in Spain was 10 / 100 000 population [8,12, 13,14].

The overall burden of CA-MRSA in Europe is the lowest in Spain and Germany (1-2%), while in countries such as Sweden and Denmark it has been reported to be between 29% and 56% [8]. The rate of MRSA remains very low in certain countries, especially the Scandinavian countries. One of the largest studies conducted over a 9-year period with 83 million person-years showed the prevalence of MRSA in Finland, Northern Denmark, Canada, Western Sweden and Canberra Australia to be 1.9/100 000. There was a higher rate of CA-MRSA of 1.0/100 000 vs 0.8/ 100 000 for HA-MRSA [15].

USA

By the end of the 1980s MRSA had replaced most *S. aureus* cases found in the hospital sector in the USA, with one study showing that 42.9% of all SAB were MRSA. A population-based study over a period ranging from 1998 – 2005 found the estimate incidence (Age and gender adjusted) of MRSA to be 12.4 per 100 000 population [16,17,18]. In the USA a multicentre study showed that CA-MRSA was higher than HA-MRSA at each centre, with rates of 76.3% and 23.6% respectively. There was an increase in CA-MRSA and inversely a decline in methicillin-sensitive staphylococcus aureus (MSSA) rates. Notably the study showed a decrease in HA-MRSA and HA-MSSA [18]. Another study carried out in Atlanta, USA, during 2005 – 2008 showed 59.4% HCA-MRSA, 26.3% HA-MRSA and 13.8% CA-MRSA [19].

Canada

A study from Canada showed an increased number of cases from 10 – 20% over the period from 2000 – 2005 with an incidence of MRSA 7.4/100 000 population and only 1% of cases were true CA-MRSA and 33% were health-care associated [13].

Australia

CA-MRSA first started making its appearance in Western Australia in 1993, and since that publication there has been an increased awareness of the prevalence of CA-MRSA [1]. A retrospective study conducted in Western Australia (Perth) over a 10-year period found that 9.6% of all cases of MRSA were pure CA-MRSA, with 52.3% being healthcare associated

(HCA-MRSA) and 38.1% HA-MRSA [20].

Asia and Pacific region

A recent meta-analysis in Asia and the Pacific region noted the highest prevalence of MRSA to be in east Asia (>40%), followed by South-East Asia (20 – 30%); the Asia-Pacific region's resistance varies between 1% and 25%. HA-MRSA was also much higher than CA-MRSA [21].

Africa

There is a paucity of literature on the epidemiology of MRSA bacteraemia in other developing countries in Africa, including the member countries of the Southern African Development Community (SADC). These studies are not exclusively based on adults nor are they all only on bacteraemia; nevertheless, the prevalence of MRSA varies between 2% and 52% [22,23]. The data on our neighbouring countries Zimbabwe and Botswana reports a prevalence of 7% and 11.2% respectively, with 44.2% of all *S. aureus* blood cultures in Botswana being associated with methicillin-resistant strains [22,23]. One of the largest studies, done in Malawi, looked at the antibiotic-resistance patterns in HA bacteraemia over eight years, and found the prevalence of MRSA to be 9.6% [24]. The lowest prevalence of MRSA was found to be in Ghana; this study revealed a very low incidence of *S. aureus* (0.6% of all blood cultures) and only one case of MRSA was identified – thus a low prevalence of 2% [25]. From the remaining African countries one study found the prevalence of MRSA to be 15%, this included the following major cities (Antananarivo, Casablanca, Dakar, Niamey and Yaounde). Data from Central Africa is limited, with Cameroon having a prevalence of between 21.3% and 28.1%.

As we move towards the Mediterranean the overall median MRSA prevalence is 39%, reaching as high as 52% in one study from Egypt [26,27,28].

Colonization of *S. aureus* has been shown to be a risk factor for the subsequent development of MRSA by the same clone. It has been shown that the pattern of colonization is similar in Africa than in Europe. Risk factors that has been established for the colonization of *S. aureus* in Africa include, HIV (Human immunodeficiency virus), frequent hand washing, living in rural areas and if hospitalized in a surgical ward. The five most common clones of MRSA in Africa is ST5-, ST8, ST80, ST88, ST239/241-MRSA. The ST8-MRSA clone has been closely related to the hypervirulent CA-MRSA clone USA300; the major CA-MRSA clone in Africa is the ST80-MRSA IV, this clone accounts for 24.2 – 88.3% of all MRSA isolates in Africa [29].

Southern Africa

Studies done between 1999 and 2003 showed that the proportion of MRSA bacteraemia in Gauteng and KwaZulu-Natal was 23% and 26.9% respectively [10,30]. A study from Perovic et al. found that CA-MRSA was about 20% of MRSA cases [10].The prevalence of MRSA bacteraemia in Gauteng was established to be around 0.03 – 0.08 cases per 1000 admissions, 38% of all isolates of SAB being methicillin resistant; this study did not focus exclusively on the adult population [31]. A larger study involving multiple provinces found that the trend for MRSA was disturbingly high, with 46% of all SAB isolates being methicillin resistant. The most number of cases was found to be in Gauteng with a prevalence of 53% [32]. Two recent studies published in 2017 looked at five hospitals from Gauteng and the Western Cape, both studies finding an incidence of MRSA of 30%. These studies showed that only 7.9% of all MRSA bacteraemia was community acquired [5,6].

1.4 Demographics of MRSA bacteraemia

Multiple international studies show that MRSA bacteraemia tends to be more common in elderly male patients. The majority of patients were above 65 years of age, and more than 57% (57– 79%) were males. One study showed the incidence of MRSA bacteraemia in elderly patients to be 133/100 000 [13,14,16,19,33, 34,35,36]. A retrospective 10-year study in Australia looking at HA-, HCA- and CA-MRSA showed that those with CA-MRSA tended to be significantly younger than those with either HA- or HCA-MRSA, at 52 years compared to 65 years [20].

Based on the South African population, two recent studies conducted in Gauteng and the Western Cape found only 19.2% of patients with MRSA bacteraemia to be above the age of 60 years in one study, with 20% being above this age in the other study, both showing a male predominance. With regard to HA-MRSA and CA-MRSA, 20.44% and 8% respectively were older than 60, again with a male predominance in both groups [5,6].

Risk factors:

The risk factors originally described for the occurrence of CA-MRSA in Australia don't seem to be noted in Europe, where they have attributed the risk to direct exposure with persons with CA-MRSA, crowded living circumstances, inadequate personal hygiene, the use of personal items between individuals and collision sports, with the highest risk factor being travel to vicinities that has been shown to have a higher prevalence of CA-MRSA [8].

Seasons:

There is some evidence of seasonality of *S. aureus*, although the evidence is based on studies on soft-tissue infections caused by *S. aureus*. One study looking at MRSA found that it was highly seasonal with hospital-acquired infections tending to peak during the winter months, while CA-MRSA tended to be more present in the summer. The seasonal variance remained true despite the different site of infections [37,38].

1.5 Definition of HA-MRSA, HCA-MRSA and CA-MRSA bacteraemia infections

Hospital-acquired bloodstream infection is defined as a positive blood culture obtained from a patient that has been admitted for 48 hours or longer. Healthcare-associated bloodstream infections are defined as a positive blood culture obtained from a patient within 48 hours after admission that meets the following criteria: recent intravenous therapy at home, wound care or specialized nursing care through a healthcare agency or self-administered intravenous medical therapy in the last 30 days before bloodstream infection; attended hospital or a haemodialysis clinic or received recent intravenous chemotherapy in the last 30 days prior to infection; recent hospitalization for two or more days in the last 90 days before bloodstream infection or resides in a nursing home/long-term facility. Community-acquired bloodstream infection is defined as a positive bloodstream infection within the first 48 hours of hospital admission that does not meet the criteria set above for a healthcare-associated infection [39].

Previous studies carried out in South Africa used slightly different definitions, and defined HA-MRSA as one occurring up to more than 72 hours after admission. The most recent study in

South Africa used the internationally accepted definition of infection within 48 hours of admission as differentiating HA-MRSA and CA-MRSA [6,10,31].

Interestingly, in the last decade the presence of livestock-associated MRSA (LA-MRSA) has also become apparent. It has been shown that LA-MRSA has been transmitted between pigs and humans as well as other animals. A recent study carried out in Australia showed the presence of highly virulent strains being transmitted between humans and pigs [40,41].

1.6 HIV infection and MRSA bacteraemia infections

Human immunodeficiency virus (HIV) infection is a global epidemic, with approximately 36.7 million people being diagnosed with HIV by the end of 2016 [42]. Approximately 19.6 million people have been diagnosed in Southern and Central Africa [43]. Infection with HIV causes a high rate of morbidity and mortality with an estimated 1 million deaths annually, the majority attributed to opportunistic infections and AIDS-defining diseases [42]. MRSA is one of the most notable opportunistic infections resulting in poor outcomes in those infected with HIV. One study found that MRSA is 6 – 18 times more likely to occur in an HIV-infected individual, with CA-MRSA three times more likely (5,6,32,42). The worldwide prevalence of MRSA has been estimated at 7% among HIV-positive patients [42].

A study carried out at an HIV clinic at Johns Hopkins Hospital in the USA found that their cohort had 94 cases of MRSA, with an absolute incidence of 11.9/1000 person years. The mean age of a person with MRSA was 42 years, and the majority were male (61.5%) [44]. The risk

factors related with the development of MRSA are hospital exposure, prior MRSA infection as well as the use of antibiotics. Other previously suggested risk factors that were not found to be significant in a recent meta-analysis included younger age, male sex, high-risk sexual activities, incarceration, low CD4+ count and high viral load, and the lack of cotrimoxazole use [42,44]. Protective factors against the development of MRSA included being on antiretroviral therapy (ART), the use of trimethoprim-sulfamethoxazole, and use of condoms [45]. The presence of HIV is an important risk factor for the development of MRSA, a study in Atlanta, Georgia, USA showed the incidence of MRSA related to HIV infection was 650/ 100 000 with 9% of their cohort having AIDS [19].

A study in the USA found that 131 out of 830 patients with SAB had HIV, the majority of these patients (66%) having MRSA and 57% of these being cases of CA-MRSA. The mean CD4+ count in these patients was 126 and only 15% patients had a suppressed viral load. There was a surprisingly low mortality rate of around 8% despite these patients being severely immunocompromised [46]. The mortality rates in HIV-positive patients are between 22% and 34%, and it remains unclear if there is an increased risk of mortality from MRSA in HIV-positive compared to HIV-negative patients [45].

An extensive review article looking at HIV and MRSA showed that the majority of infections in HIV-positive individuals were of the skin and soft tissue (85%). MRSA bacteraemia in HIV-infected individuals has been shown to be less since the introduction of ART; nonetheless there is a 16-fold increased risk associated with the development of MRSA [45].

HIV infection is an important risk factor for the development of MRSA, as documented in both local and international studies. The presence of a high prevalence of HIV infection in Southern Africa makes it especially important to look at the presence of MRSA in our population.

1.7 MRSA bacteraemia infections and co-morbidities

The Charlson co-morbidity index was used in a few studies. This score is a weighted index that was devised in 1987 and has been used to quantify the impact of a particular co-morbid condition on a specific patient. The score consists of a subgroup of categories with each category being assigned a score; a higher score is associated with a poorer outcome. Mortality increases with the Charlson index, with a score of 0 being related to a 14% mortality and a score > 3 related to a 31% mortality [13,16,47].

Pre-existing conditions / co-morbid conditions associated with MRSA include the following: diabetes mellitus, chronic renal failure, haemodialysis / end-stage renal disease, major surgery, congestive cardiac failure, cerebrovascular disease, coronary artery disease, malignancy (solid tumours / haematological), immunosuppression, steroid use, liver cirrhosis, implanted devices / foreign bodies, transplant, AIDS, and chronic liver disease [13,14,16,19,33, 34,35,36].

1.8 Common site of MRSA infections

The clinical syndromes that are associated with MRSA include endocarditis, pneumonia, infections of skin and soft tissue, bone and joint infections (septic arthritis and osteomyelitis), catheter-related and surgical wound infections and other. The most common sites mentioned in the literature are skin and soft tissue, catheter-related, bone and joint infections, endocarditis and pneumonia [39].

The infections associated with CA-MRSA are almost always of skin and soft tissue, and are responsible for 90% of the cases. These skin infections may be moderately severe too severe with occasional severe skin infections such as necrotizing fasciitis. The remaining sites of infection include bones and joints and the respiratory and urogenital systems [7].

A large study looking at the potential sites of invasive MRSA in patients between 2005 and 2008 concluded that in 45% of patients with HA-MRSA and 60% of patients with HCA-MRSA the only site of MRSA was blood cultures. However in HA-MRSA bacteraemias, pneumonia (18%) was the second most common site and in HCA-MRSA other common sites where bone and joint infections (13%), pneumonia (12%) and skin and soft tissue infections (12%) [48].

1.9 Outcomes of MRSA bacteraemia infections

MRSA bacteraemia has been shown to prolong hospital stay when compared to methicillin-susceptible *S. aureus*. In one study the median hospital stay was prolonged by 7.5 days for SAB, and there was a 1.29-fold increase in hospital stay in MRSA compared to MSSA (48). The mortality rate of patients with MRSA bacteraemia varied from 23.5% to 58% in different

studies in the literature [9,13,14,16,19,33, 34,35,36]. Significant risk factors associated with a high 14-day mortality rate in patients with *S. aureus* bacteraemia: admission to the intensive care unit (ICU), inotropic support, intubated patients, organ dysfunction, pyogenic complications, surgery and intravenous catheters [10].

1.10 *Treatment of MRSA bacteraemia infections*

The management of MRSA bacteraemia is very important as it has been linked to a high mortality rate. One study showed that the most crucial facet of MRSA management is the timely identification of MRSA [36]. The choice of antibiotic is driven by susceptibility testing. The main antibiotics of choice used in the treatment of MRSA are the glycopeptides, especially vancomycin [13]. Occasionally rifampicin and gentamycin have been added to the treatment regimen, but the evidence does not support the use of combination therapy.

The current suggested management of uncomplicated MRSA bacteraemia (classified as a positive blood culture and no evidence of infective endocarditis, no implanted prosthesis, subsequent blood cultures negative at 48 – 96 hours, no fever after 72 hours and no evidence of disseminated infection) is vancomycin or daptomycin 6 mg/kg/dose intravenously daily for a minimum of two weeks. Patients with complicated bacteraemia (classified as a positive blood culture which does not meet the criteria for uncomplicated bacteraemia) vancomycin or daptomycin for 4–6 weeks, with some advocating a higher dosage of daptomycin at 8 mg/kg/dose [50,51].

Effective treatment is using an antibiotic to which the organism has been tested to be susceptible and then administrating that antibiotic for at least seven days of the initial two weeks. In this study 48% was started on effective treatment within the first 24 hours of culture [16]. Empiric antibiotic treatment is defined as when an antibiotic is started after the blood culture has been taken. In one study only 13 of 51 patients received adequate empiric antibiotics. Antibiotics that were given empirically included the following: cephalosporin (29 out of 51), vancomycin (8 out of 51), piperacillin-tazobactam (8 out of 51), clindamycin (2 out of 51), linezolid (4 out of 51) and daptomycin (1 out of 51) [33].

Aim

MRSA is a serious infection with increased mortality, increased hospital stays and increased hospital costs. Recently CA-MRSA has been on the rise. This study aims to describe the differences between MRSA in our setting and that of available literature available and to delineate between community and hospital acquired MRSA.

Study Objectives

Primary objectives

1. To describe the clinical and demographic characteristics of patients presenting with MRSA bacteraemia.

2. To compare patients presenting with CA-MRSA to HA-MRSA bacteraemia.

Secondary objectives

1. To identify any additional risk factors associated with mortality in patients with MRSA bacteraemia.
2. To determine the proportion of patients with MRSA infection and HIV co-infection.
3. To evaluate the effective use of antibiotics in patients presenting with MRSA bacteraemia.

Methods

Setting

The study will take place at Chris Hani Baragwanath Academic Hospital (CHBAH). It is the third largest hospital in the world, and it cares mainly for the community of Soweto in Johannesburg. It consists of approximately 3200 beds. The hospital forms part of one of the academic hospitals of the University of the Witwatersrand.

Study population

The study population will consist of adult patients admitted to CHBAH that had a MRSA isolate on blood culture, during the period 1 Jan 2013 to 31 Dec 2015. If the required sample size is not achieved during this period, I will extend the study one year earlier or until the required sample size have been achieved. These patients mostly reside in Soweto, with the majority being black African patients from low-middle socio-economic status. The known prevalence of MRSA bacteraemia in adults at CHBAH is unknown, therefore the sample size required for this study was calculated based on the known prevalence of MRSA in Gauteng by a study done by Perovic et.al in 2006 [10].

Study Design

This will be a retrospective cross-sectional observational study.

Data collection

The patients will be identified by looking at the blood culture isolate database from the National Health Laboratory Service (NHLS) at CHBAH.

Inclusion criteria:

- All patients older than 18 years of age with a positive blood culture showing MRSA during the study period will be included in the study.

Exclusion criteria:

- Any patient that was younger than 18 years of age.
- Patients that had signs and symptoms of sepsis on admission and has not had a blood culture taken on admission.
- Patients in which the records were missing to a point where it was not possible to classify patients as HA-MRSA, CA-MRSA or HCA-MRSA.

The hospital number as well as the patient's name and surname will be used to trace the patient's records, but the patient's details will be kept anonymous by delinking the results once collected. A request will be submitted to the records department to retrieve the file of the patient.

Data from the file will be transferred to a data collection sheet (see Appendix A). Data collected will include the following:

- Demographics:
    - Age
    - Gender
    - Race
- Department: Medical, Surgical, Obstetrics and Gynaecology, Psychiatry.
- Hospital stay and history:
    - Date of admission

- Outcome: Discharged Alive, Mortality ≤ 14 days, Mortality ≤ 14 days, Mortality > 30 days.
- Length of hospital stay in days
- Admission to ICU
- Recent hospital admission for more than two days in the last 90 days
- Haemodialysis
- Nursing home resident
- Intravenous chemotherapy
- Previous MRSA
- Admission diagnosis

- Blood culture:
  - Date of culture, isolate and sensitivity
- Site of infections
- Laboratory values:
  - White cell count ($x10^9$/l)
  - Platelets ($x10^9$/l)
  - C reactive protein (mg/l)
  - Procalcitonin
  - Glomerular filtration rate (Estimate GFR)
  - Total bilirubin (umol/l)

- Albumin (g/l)
- International normalized ratio
- Alanine aminotransferase (U/l)
- Asparate aminotransferase (U/l)
- Alkaline phosphatase (U/l)
- Gamma-glutamyl-trasnferase (U/l)

- Clinical characteristics and SOFA Score
  - Temperature oC
  - Heart rate (BPM)
  - Respiratory rate (rate per minute)
  - Systolic blood pressure (mmHg)
  - Mean arterial pressure (mmHg)
  - P/F ratio
  - Glascow coma scale
- Co-morbidities classified according to Charlson co-morbidity index (See below)
- Risk factors for mortality
- HIV
  - Status
  - CD4+ count, on ART, VL Suppressed
- Antibiotic use

It is important to note that co-morbidities are powerful predictors of hospital outcomes and therefore in this study the Charlson co-morbidity index was used to assess the co-morbidities of our study. This index originally described in 1987 was developed by Charlson in an attempt to prognosticate the 1year mortality based on the burden of disease in patients admitted to hospital it is based on 17 diagnoses each weighted by mortality risk. The Charlson score has been subsequently validated in a variety of large populations. A large systematic review using a Canadian administrative database concluded that the use of the Charlson index for the use with administrative databases discriminates mortality equally [47, 52, 53, 54].

The SOFA (Sequential organ failure assessment) risk score will also take into account the severity of the illness the patient currently has. This scoring system is commonly used in critical care to assess the severity of illness. The SOFA score was designed as research tools to predict which patients with sepsis were likely to die. The SOFA score is a simple and effective way to describe organ dysfunction in critically ill patients [55].

The clinical and laboratory characteristics were obtained on the date of admission and when the blood culture was done. The blood cultures were taken on admission by NHLS phlebotomists using a sterile technique.

Any missing data not in the file will be supplemented by going through the patient's results using Trak care system. This will be helpful in identifying any possible source of the bacteraemia such as pus swabs from wounds, catheter tips, urine microscopy and drug levels. Data will be transferred to an Excel spread sheet and from there it will be transferred to Statistica to analyze the data.

Data Analysis

To achieve a power at 95% confidence interval a sample size of +- 100 - 120 will be required.

$n= (Z_{2}1\text{-}a\ P\ (1\text{-}P))/D_{2}$

$n= (1.96_{2}*0.08(1\text{-}0.08))/0.05_{2}$

$n= (1.96_{2}*0.0736)/0.05_{2}$

n=0.28274176/0.0025

n=113

The sample size was calculated based on study done by Fortuin-de Smidt et.al in which the prevalence of MRSA was calculated at 0.08 [31]. This number was used in this study to calculate the sample size needed to have a confidence interval of 95%. To calculate the difference between community versus hospital acquired MRSA descriptive statistics will be used. If age is normally distributed the Student T-Test will be used, if not normally distributed the Mann-Whitney test will be used. Univariant analysis using the Chi-squared test will be used for the remaining categorical data if assumptions are met, if the assumptions are not met the Fischer's exact test will be used instead. The *p* value will be set at <0.05 at a confidence interval of 95% for statistical significance. Finally, multivariant analysis using logistical regression will be used to test for any confounders.

Ethics

Ethical approval will be sought from the Human Research Ethics Committee of the University of the Witwatersrand. Only the primary researcher will have access to the data set. Permission to conduct the research will also be sought from the management of CHBAH as well as from the NHLS. All data that will be collected will remain anonymous and no patient personal details will be used in the study to maintain confidentiality. As the study is retrospective there will be no risk to any patient that is involved in the study

Timing

	Jan	**Feb**	**Mar**	**April**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan 17**	**Feb**	**Mar**	**April**	**May**	**June**	**Jun**	**Aug**
Literature review																				
Preparing Protocol																				
Protocol Assessment																				
Ethics Application																				
Collecting Data																				

Data Analysis																		
Writing up – Thesis																		
Writing up – Paper																		

Funding

The only expenses for this research will be printing costs, internet cost and stationery. These costs will be covered by me.

Limitations

The limitations that may be encountered in this study are duplicate cultures results, missing patient records, poor note keeping and the availability of drugs. This study also includes mainly African black patients and may not be applicable to the rest of the South-African population in the health care sector.

References – (Extended Literature Review)

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant Staphylococcus aureus as a public-health threat. Lancet. 2006; 368(9538):874–85.

2. Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol. 2008 Dec; 8(6):747–63.

3. Hübner C, Hübner NO, Hopert K, Maletzki S, Flessa S. Analysis of MRSA-attributed costs of hospitalized patients in Germany. Eur J Clin Microbiol Infect Dis. 2014; 33(10):1817–22.

4. Lowy F. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003; 111(9):1265–73.

5. Shuping LL, Kuonza L, Musekiwa A, Iyaloo S, Perovic O. Hospital-associated methicillin-resistant Staphylococcus aureus: A cross-sectional analysis of risk factors in South African tertiary public hospitals. PLoS One. 2017; 12(11):1–14.

6. Perovic O, Singh-Moodley A, Govender NP, Kularatne R, Whitelaw A, Chibabhai V, et al. A small proportion of community-associated methicillin-resistant Staphylococcus aureus bacteraemia, compared to healthcare-associated cases, in two South African provinces. Eur J Clin Microbiol Infect Dis. 2017; 36(12):2519–32.

7. Otto M. Community-associated MRSA: What makes them special? Int J Med Microbiol. 2013; 303(6–7):324–30.

8. Köck R, Becker K, Cookson B, van Gemert-Pijnem JE, Harbarth S, Kluytmans JAJW, et al. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 2010; 15(41):1–9.

9. Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA). Curr Opin Microbiol. 2012; 15(5):588–95.

10. Perovic O, Koornhof H, Black V, Moodley I, Duse A, Galpin J. Staphylococcus aureus bacteraemia at two academic hospitals in Johannesburg. S Afr Med J. 2006; 96(8):714–7.

11. Frimodt-Møller N, Espersen F, Skinhøj P, Rosdahl VT. Epidemiology of Staphylococcus aureus bacteremia in Denmark from 1957 to 1990. Clin Microbiol Infect. 1997; 3(3):297–305.

12. Tiemersma EW, Bronzwaer SLAM, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N, et al. Methicillin-resistant Staphylococcus aureus in Europe, 1999-2002. Emerg Infect Dis.2004; 10(9):1627–34.

13. Gasch O, Ayats J, Angeles Dominguez M, Tubau F, Liñares J, Peña C, et al. Epidemiology of Methicillin-Resistant Staphylococcus aureus (MRSA) Bloodstream Infection: Secular Trends Over 19 Years at a University Hospital. Medicine (Baltimore). 2011; 90(5):319–27.

14. Wyllie DH, Peto TE a, Crook D. MRSA bacteraemia in patients on arrival in hospital: a cohort study in Oxfordshire 1997-2003. BMJ. 2005; 331:992.

15. Laupland KB, Lyytikäinen O, Søgaard M, Kennedy KJ, Knudsen JD, Ostergaard C, et al. The changing epidemiology of Staphylococcus aureus bloodstream infection: A

multinational population-based surveillance study. Clin Microbiol Infect. 2013; 19(5):465–71.

16. Allard C, Carignan A, Bergevin M, Boulais I, Tremblay V, Robichaud P, et al. Secular changes in incidence and mortality associated with Staphylococcus aureus bacteraemia in Quebec, Canada, 1991–2005. Clin Microbiol Infect. 2008; 14:421–8.

17. El Atrouni WI, Knoll BM, Lahr BD, Eckel-Passow JE, Sia IG, Baddour LM. Temporal Trends in the Incidence of Staphylococcus aureus Bacteremia in Olmsted County, Minnesota, 1998 to 2005: A Population-Based Study. Clin Infect Dis. 2009; 49:130–8.

18. David MZ, Daum RS, Bayer AS, Chambers HF, Fowler VG, Miller LG, et al. Staphylococcus aureus bacteremia at 5 US Academic Medical Centers, 2008-2011: Significant geographic variation in community-onset infections. Clin Infect Dis. 2014; 59(6):798–807.

19. Kempker RR, Farley MM, Ladson JL, Satola S, Ray SM. Association of methicillin-resistant Staphylococcus aureus (MRSA) USA300 genotype with mortality in MRSA bacteremia. J Infect. 2010; 61(5):372–81.

20. Robinson JO, Pearson JC, Christiansen KJ, Coombs GW, Murray RJ. Community-associated versus healthcare-associated methicillin-resistant Staphylococcus aureus bacteraemia: A 10-year retrospective review. Eur J Clin Microbiol Infect Dis. 2009; 28(4):353–61.

21. Lim WW, Wu P, Bond HS, Wong JY, Ni K, Seto WH, et al. Determinants of MRSA prevalence in the Asia Pacific Region: a systematic review and meta-analysis. J Glob Antimicrob Resist. 2018, forthcoming.

22. Wood SM, Shah SS, Bafana M, Ratner AJ, Meaney PA, Malefho KCS, et al. Epidemiology of methicillin-resistant Staphylococcus aureus bacteremia in Gaborone, Botswana. Infect Control Hosp Epidemiol. 2009; 30(8):782–5.

23. Mauchaza K, Madzimbamuto FD, Waner S. Methicillin-resistant Staphylococcus aureus in Zimbabwe. Ghana Med J. 2016; 50(2):68–71.

24. Musicha P, Cornick JE, Bar-Zeev N, French N, Masesa C, Denis B, et al. Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. Lancet Infect Dis. 2017; 17(10):1042–52.

25. Dekker D, Wolters M, Mertens E, Boahen KG, Krumkamp R, Eibach D, et al. Antibiotic resistance and clonal diversity of invasive Staphylococcus aureus in the rural Ashanti Region, Ghana. BMC Infect Dis. 2016; 16(1):1–6.

26. Breurec S, Zriouil SB, Fall C, Boisier P, Brisse S, Djibo S, et al. Epidemiology of methicillin-resistant Staphylococcus aureus lineages in five major African towns: Emergence and spread of atypical clones. Clin Microbiol Infect. 2011; 17(2):160–5.

27. Huson MAM, Kalkman R, Remppis J, Beyeme JO, Kraef C, Schaumburg F, et al. Methicillin-resistant Staphylococcus aureus as a cause of invasive infections in Central Africa: A case report and review of the literature. Infection. 2014; 42:451–7.

28. Borg MA, de Kraker M, Scicluna E, van de Sande-Bruinsma N, Tiemersma E, Monen J, et al. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in invasive isolates from southern and eastern Mediterranean countries. J Antimicrob Chemother. 2007; 60(6):1310–5.

29. Schaumburg F, Alabi A, Peters G, Becker K. New epidemiology of *Staphylococcus aureus* infection in Africa. Clin Microbiol Infect.2014; 20(7): 589 – 596.

30. Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in KwaZulu-Natal province, South Africa. BMC Infect Dis. 2006; 6:125.

31. Fortuin-de Smidt MC, Singh-Moodley A, Badat R, Quan V, Kularatne R, Nana T, et al. Staphylococcus aureus bacteraemia in Gauteng academic hospitals, South Africa. Int J Infect Dis. 2015; 30:41–8.

32. Perovic O, Iyaloo S, Kularatne R, Lowman W, Bosman N, Wadula J, et al. Prevalence and Trends of Staphylococcus aureus Bacteraemia in Hospitalized Patients in South Africa, 2010 to 2012: Laboratory-Based Surveillance Mapping of Antimicrobial Resistance and Molecular Epidemiology. PLoS One. 2015; 10(12):1 - 14.

33. Rahikka P, Syrjanen J, Vuento R, Laine J, Huttunen R. Meticillin-resistant Staphylococcus aureus (MRSA) bacteraemia in Tampere University Hospital: a case-control study, Finland October 2002 to January 2010. Eurosurveillance. 2011; 16(35):17–21.

34. Van Cleef BAGL, Kluytmans JAJW, van Benthem BHB, Haenen A, Monen J, Daniels-Haardt I, et al. Cross border comparison of MRSA bacteraemia between The Netherlands and North Rhine-Westphalia (Germany): a cross-sectional study. PLoS One. 2012; 7(8):e42787.

35. Chen L-Y, Chen L-K, Chang C-W, Kuo S-C, Li L-F, Chan Y-J, et al. Treatment of community-onset methicillin-resistant Staphylococcus aureus (MRSA) bacteremia: a hospital-based study. Arch Gerontol Geriatr. 2012; 55(1):152–6.

36. Forstner C, Dungl C, Tobudic S, Mitteregger D, Lagler H, Burgmann H. Predictors of clinical and microbiological treatment failure in patients with methicillin-resistant

Staphylococcus aureus (MRSA) bacteraemia: a retrospective cohort study in a region with low MRSA prevalence. Clin Microbiol Infect. 2013; 19(7):291-7.

37. Leekha S, Diekema DJ, Perencevich EN. Seasonality of staphylococcal infections. Clin Microbiol Infect. 2012; 18(10):927–33.

38. Klein EY, Sun L, Smith DL, Laxminarayan R. The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: A national observational study. Am J Epidemiol. 2013; 177(7):666–74.

39. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management of Osteoarticular Infections in Children. Clin Microbiol Rev. 2015; 28(3):603 – 661.

40. Bi Z, Sun C, Börjesson S, Chen B, Ji X, Berglund B, et al. Identical genotypes of community-associated MRSA (ST59) and livestock-associated MRSA (ST9) in humans and pigs in rural China. Zoonoses Public Health. 2018; November 2017:1–5.

41. Sahibzada S, Abraham S, Coombs GW, Pang S, Hernández-Jover M, Jordan D, et al. Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. Sci Rep. 2017; 7(1):1–11.

42. Sabbagh P, Riahi SM, Gamble HR, Rostami A. The global and regional prevalence, burden, and risk factors for methicillin-resistant Staphylococcus aureus colonization in HIV-infected people: A systematic review and meta-analysis. Am J Infect Control. 2018;00; 1 - 11.

43. Secretary's Minority AIDS Initiative Fund(SMAIF)[Internet]. Global Statistics. SMAIF; [updated 2018 Nov 20]. Available from: https://www.hiv.gov/hiv-basics/overview/data-and-trends/global-statistics.

44. Burkey MD, Wilson LE, Moore RD, Lucas GM, Francis J, Gebo KA. The incidence of and risk factors for MRSA bacteraemia in an HIV-infected cohort in the HAART era. HIV Med. 2008; 9(10):858–62.

45. Shadyab AH, Crum-Cianflone NF. Methicillin-resistant Staphylococcus aureus (MRSA) infections among HIV-infected persons in the era of highly active antiretroviral therapy: A review of the literature. HIV Med. 2012; 13(6):319–32.

46. Furuno JP, Johnson JK, Schweizer ML, Uche A, Stine OC, Shurland SM, et al. Community-associated Methicillin-resistant Staphylococcus aureus Bacteremia and Endocarditis among HIV Patients: A cohort study. BMC Infect Dis. 2011; 11(1):298.

47. Charlson ME, Pompei P, Ales KL, Mackenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J Chron Dis. 1987; 40(5):373–83.

48. Kallen AJ, Mu Y, Bulens S, Reingold A, Petit S, Gershman K, et al. Health care-associated invasive MRSA infections, 2005-2008. JAMA. [Internet]. 2010; 304(6):641–8.

49. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol. 2005; 26(2):166–74.

50. Cosgrove SE, Fowler, Jr. VG. Management of Methicillin-Resistant Staphylococcus aureus Bacteremia. Clin Infect Dis. 2008; 46(S5):S386–93.

51. Fowler V, Saglio G, Wook Kim D, Issaragrisil S, Le Courtre P, Etienne G, et al. Daptomycin versus Standard Therapy for Bacteremia and Endocarditis Caused by Staphylococcus aureus. N Engl J Med. 2006; 362(24):2251–9.

52. Sundararajan V, Henderson T, Perry C, Muggivan A, Quan H, Ghali A. New ICD-10 version of the Charlson Comorbidity Index predicted in-hospital mortality. J Clin Epidemiol. 2004; 57: 1288 – 1294.

53. Gabriel S, Crowson C, O'Fallon M. A comparison of two co-morbidity instruments in Arthritis. J Clin Epidemiol. 1999; 52(12): 1137-1142.

54. Needham D, Scales D, Laupacis A, Pronovost P. A systematic review of the Charlson co-morbidity index using Canadian administrative databases: a perspective on risk adjustments in critical care research. J Crit Care. 2005; 20: 12-19.

55. Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM et. al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine.1998; 26(11): 1793 – 800.

Chapter 2: Manuscript

Title:

Methicillin-Resistant Staphylococcus aureus bacteraemia in adults at Chris Hani Baragwanath Academic Hospital.

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Conflict of interest:

None

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Word count:

Total: 4293 Words

Abstract: 302 Words

Abstract

Introduction: The advent of methicillin-resistant Staphylococcus aureus (MRSA) has led to poor patient outcomes and longer hospital stays. Initially described as a nosocomial infection, it has now led to the development of community-acquired (CA) strains. This study aimed to describe the differentiation between CA and hospital-acquired (HA) MRSA infections.

Methods: A retrospective study was conducted at Chris Hani Baragwanath Academic Hospital in Johannesburg from 1 January 2013 to 31 December 2015. Cases were detected utilising the National Health Laboratory Service. One hundred adult patients that met the inclusion criteria were included in the study. Descriptive statistics was used using the Pearson chi-squared test and a p value of less 0.05 with a confidence interval of 95% was used as statistically significant.

Results: Seventy-seven cases were HA, 5 cases were CA and 18 cases were healthcare-associated (HCA). The all-cause mortality was not statistically different between the three groups, but the all-cause mortality was higher in HIV positive patients (61% vs 38%, p value <0.05). Forty-two of 43 cases in the surgical department were HA (97.7% vs. 2.3%); 4 of 5 cases of CA-MRSA and all 18 cases of HCA-MRSA were medical patients. Thirty-nine patients (39 %) were admitted to the intensive care unit. Fifty-seven patients (57%) were HIV-positive, and 34 (60%) were on antiretroviral therapy. Soft-tissue (24%) and catheter-associated infections (21%) were the two most common sources of infection in HA-MRSA patients. HA-MRSA had a lower Charlson co-morbidity index compared to both HCA-MRSA and CA-MRSA (3 vs 6 and 6 respectively, p< 0.05).

Conclusion: Rates of CA-MRSA infections remain low in our setting compared to international data, but may be underestimated due to the small sample size. While HA-MRSA is common in surgical patients, HCA-MRSA makes up the majority of cases in medical patients, with co-morbidities and previous hospital exposure important determinants.

Introduction

Staphylococcus aureus (S. aureus) a highly virulent human commensal with the ability to result in a wide spectrum of diseases, from minor skin infections to multiple life-threatening infections [1,2]. The development of methicillin-resistant *S. aureus* (MRSA) strains has led to poor patient outcomes, longer hospital stays and subsequent increased economic burden [3]. The development of resistance is attributed somewhat to the inappropriate and indiscriminate use of antimicrobials not only by the healthcare sector but also the agricultural sector, and globalization with the resultant increase in international travel has led to swift spread across international borders [4].

While MRSA was initially only attributed to nosocomial infections, it has now spread into the community resulting in infections in patients who have recent as well as no prior hospital exposure [4]. In a large multicentre study in the USA there was an increase in community-acquired MRSA ((CA)-MRSA) with 76.3% being CA-MRSA and 23.6% being hospital-acquired MRSA ((HA)-MRSA) [5].

The prevalence of MRSA bacteraemia in Gauteng was established to be around 0.03 – 0.08 cases per 1000 admissions with two recent studies looking at five hospitals in Gauteng and the western cape establishing the rate of MRSA bacteraemia at 30% with only 7.9% of these cases being CA-MRSA [6 – 8]. The data on MRSA bacteraemia at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg, is limited. It is therefore important to study this bacterium in our setting and to compare (HA)-MRSA to (CA)-MRSA to ensure that adequate preventative and treatment modalities can be implemented based on our local features of *S. aureus.*

Methods

Study Design and Population

This was a retrospective cross-sectional observational study conducted at CHBAH, the third largest hospital in the world with approximately 3200 beds, 150 000 in-patients and 500 000 out-patients per year. It is located in Soweto, Johannesburg, and caters mostly for African patients of low socio-economic status. The study was conducted over a three-year period from 1 January 2013 to 31 December 2015. All adult patients older than 18 years with a positive MRSA isolate on blood culture were identified using the corporate data warehouse (CDW) at the National Health and Laboratory Service (NHLS).The following exclusion criteria were applied: (1) patients younger than 18 years; (2) if the patient had sepsis on admission, but no blood culture was done; and (3) those with an incomplete file.

Case Definitions

A case was defined as having either hospital-acquired MRSA (HA-MRSA), community-acquired MRSA (CA-MRSA) or healthcare-associated MRSA (HCA-MRSA). A patient with a positive blood culture isolate of MRSA $\geq$ 48 hours after admission was defined as having HA-MRSA. The second group consisted of those with a positive blood culture < 48 hours after admission and was subsequently divided into those with CA-MRSA and those with HCA-MRSA. If there was no prior healthcare exposure in the preceding three months, the patient was said to have CA-MRSA. If the patient met any of the following criteria they were labelled as HCA-MRSA: (1) recent intravenous therapy at home, wound care or specialized nursing care through a healthcare agency or self-administered intravenous medical therapy in the last

30 days before bloodstream infection; (2) attended hospital or a haemodialysis clinic or received recent intravenous chemotherapy in the last 30 days prior to infection; (3) recent hospitalization for 2 or more days in the last 90 days before bloodstream infection; and (4) resides in a nursing home/long-term facility.

Data Collection

A case was identified using the NHLS CDW. The patient's clinical records were accessed from the records department at CHBAH and additional laboratory results were accessed using NHLS TrakCare Lab Webview. The admission records using Medtronic as well as TrakCare were used to identify prior hospital admissions. The clinical and laboratory information obtained was transferred onto a standardized data collection sheet and subsequently transferred to Excel.

Statistical Analysis

The desired sample size needed was calculated using available epidemiological data and was found to be 113 (100–120). The cases were grouped into three groups: HA-MRSA, CA-MRSA or HCA-MRSA and compared. The variables considered at were gender, race, department, season, outcome, site of infection, risk factors for mortality, Human-immunodeficiency-virus (HIV), clinical data, SOFA (Sepsis-related organ failure assessment) score on admission and culture, Charlson score and co-morbidities, and antibiotic use. Descriptive statistics were applied to demographic and clinical/laboratory characteristics using Pearson's chi-squared test. A p value of <0.05 was used for statistical significance with a confidence interval of 95%. Proportions were also calculated, dividing the number of each group into the total number of cases. A second model was run using multivariate analysis on risk factors for mortality (9,10).

Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (Protocol No: M160925). Permission to conduct the research was also obtained from the management of CHBAH as well as from the NHLS.

Results

A total of 559 cases were identified during the study period 1 January 2013 – 31 December 2015; of these, 335 were in patients younger than 18 years and excluded. From the remaining 224 patients, 124 (55%) were excluded either due to missing records or inability to reliably differentiate between HA-MRSA or CA-MRSA. Seventy-seven cases (77%) were regarded as HA-MRSA, 18 cases (18%) were HCA-MRSA and 5 cases (5%) were CA-MRSA (see Figures 1 and 2).

Demographics

The patients were predominantly male (59%), with no statistically significant difference between the three groups, X_2=0.7 (df=2, N=100, p <0.05), with 61% (n=47) for HA-MRSA, 50% (n=9) in HCA-MRSA and 60% (n=3) in CA-MRSA. There was no statistically significant difference between median age in the different groups (H(2)=3.4, p=0.184), with a median age of 35 (IQR 21) for HA-MRSA, 47 (IQR 22) for HCA-MRSA and 45 (IQR 9) for CA-MRSA (see Figure 3). The majority of patients (92%) were African.

Hospital Stay

There was a statistically significant difference between the numbers of patients admitted to the medical department in the three groups X_2 =19.7 (df=2, N=100, p <0.05), with 44% (n=34) for HA-MRSA, 100% (n=18) for HCA-MRSA and 80% (n=4) for CA-MRSA. There was a statistically significant difference between the number of patients admitted to the surgical department in the three groups X_2=18.9 (df=2, N=100, p <0.05), with 55% (n=42) for HA-MRSA, 0% (n=0) for HCA-MRSA and 20% (n=1) for CA-MRSA. There was a statistically significant difference between the overall length of hospital stay and the different groups (H(2)=32.1, p<0.05), with a median stay of 30 days (IQR 26) for HA-MRSA, 8 days (IQR 8) for HCA-MRSA and 7 days (IQR 20) for CA-MRSA.

There was no statistically significant difference between the length of hospital stay after culture and the different groups (H(2)=0.96, p=0.620), with a median stay of 13 days (IQR 19) for HA-MRSA, 8 days (IQR 8) for HCA-MRSA and 6 days (IQR 20) for CA-MRSA. The overall regression model was significant (F(3,96)=4.3, p<0.05, R_2 =0.1). Taken as a set, the predictors between the three groups and the number of days from culture to outcome account for 12% of the variance in outcome. There was no statistical difference between the number of days and the outcome between HA-MRSA, HCA-MRSA and CA-MRSA, but there was a negative correlation between the number of days to outcome (R= -0.01, p <0.05).

For patients admitted to ICU there was a statistically significant difference between the three groups X_2=19.1 (df=2, N=100, p <0.05), with all patients being from the HA-MRSA group (51%, n=39).Twenty-four (31%) of the HA-MRSA and 100% (n=18) of the HCA-MRSA group had a recent admission within the last 90 days, and this was statistically significant X_2 =

32.2 (df=2, N=100, p <0.05). The reasons for admission are shown in Table 3. In 52% (n=40) of cases of HA-MRSA these were related to trauma, with 88% (n=35) due to burns. There was a statistically significant difference between burns in the three groups X_2 = 16.1 (df=2, N=100, p <0.05), with 45% (n=35) in HA-MRSA cases, 0% (n=0) in HCA-MRSA and 0% (n=0) in CA-MRSA. There was a statistically significant difference between tuberculosis in the three groups X_2 = 7.1 (df=2, N=100, p <0.05), with 18.9% (n=13) in the HA-MRSA group, 44.4% (n=8) in HCA-MRSA and 40% (n=2) in CA-MRSA.

Blood Cultures

Twenty-seven (27%) blood cultures were polymicrobial, from these blood cultures with more than one isolate, 41% (n=11) had an additional culture confirming MRSA isolate (central line being the most common). From the remaining 16 polymicrobial blood cultures, 14 (88%) had median CRP of 206 mg/L (IQR 181, 61–491 mg/L) at the time of blood culture and two (13%) had no CRP at the time of culture. In these two cases with no CRP, one was treated once culture became available and the other died before the result became available.

Site of infection

There was no statistically significant site or additional culture between the three groups. A likely cause of infection was isolated in 60% (n=46) of HA-MRSA, 60% (n=3) of CA-MRSA and 39% (n=7) of HCA-MRSA cases. Skin and soft tissue were the most common site in HA-MRSA (24.4%, n=19) with 20% (n=1) in CA-MRSA and 5.6% (n=1) in HCA-MRSA. Intravascular catheter-associated infection was seen in 21.8% (n=17) of HA-MRSA cases and 11.1% (n=2) of HCA-MRSA cases. Pneumonia was the most common presentation in CA-

MRSA (40%, n=2), with 10.3% (n=8) in HA-MRSA and 11.1% (n=2) in HCA-MRSA. There were 34 cultures other than blood cultures that revealed MRSA. Thirty-two cultures were HA-MRSA; these include central line (n=16, 20.8%), pus swab (n=8, 10.4%), tissue (n=3, 3.90%) (see Figure 4). HCA-MRSA was only found on two pus swabs (11.1%) (see Table 3).

Clinical and laboratory characteristics

There was no statistical difference between clinical features at the time of culture in the three groups (see Table 2). There was a statistical difference between the white cell count at the time of culture in the three groups: 12.8 x 10^9/L(IQR 11.33) in HA-MRSA, 7 x 10^9/L((IQR 6) in HCA-MRSA and 4.3 x 10^9/L((IQR 1.62) in CA-MRSA ($H(2)=10.5$, $p < 0.05$). There was a statistically significant difference between the CRP in the three groups: the median CRP was 200 mg/L (IQR 166) in HA-MRSA, 164 mg/L (IQR 161) in HCA-MRSA and 108 mg/L (IQR 112) in CA-MRSA ($H(2)= 5.3$, $p<0.05$). There was no statistically significant difference in the remaining laboratory features in the three groups (see Table 1). The clinical severity score SOFA was statistically different in the three groups, with a median score of 4 (IQR 3) in HA-MRSA, 4 (IQR 3, 1–9) in HCA-MRSA and 1 (IQR 1, 1–2) in CA-MRSA ($H(2)$, $p<0.05$).

Co-morbidities

Patients with HA-MRSA had a lower Charlson co-morbidity index compared to both HCA-MRSA and CA-MRSA (3 vs 6 and 6 respectively, $p< 0.05$). The most common co-morbidities in HA-MRSA were HIV/AIDS (40%, n=31), moderate or severe renal failure (35%, n=27), mild liver disease (26%, n=20), and cerebrovascular disease (5%, n=4). In HCA-MRSA the most common co-morbidities were HIV/AIDS (67%, n=12), moderate or severe renal failure

(44%, n=8), mild liver disease (17%, n=3), diabetes mellitus (6%, n=2), and dementia (6%, n=2). In CA-MRSA the most common co-morbidities were HIV/AIDS (80%, n=4), mild liver disease (20%, n=1) and lymphoma (20%, n=1).

Outcomes

There was no statistical difference in the all-cause mortality between the three groups: the mortality rate was 53% (n=41) in HA-MRSA, 67% (n=12) in HCA-MRSA and 40% (n=2) in CA-MRSA, but there was a significant difference between the 14-day mortality in the three groups, with 8% (n=6) in HA-MRSA, 56% (n=10) in HCA-MRSA and 20% (n=1) in CA-MRSA $X_2 = 23.62$ (df=2, N=17, $p < 0.05$). The > 30-day mortality was almost statistically significant in the HA-MRSA group, with 21% (n=16) in HA-MRSA and 0% in the other two groups $X_2 = 5.57$ (df=2, N=16, p=0.058).Only HA-MRSA patients were admitted to the ICU and there was a statistical difference between patients admitted to ICU and the outcome, mortality being 49% (n=19) in patients admitted to ICU compared with 58% (n=22) in patients not admitted to ICU $X_2 = 6.1$ (df=1, N=77, $p < 0.05$). The all-cause mortality was statistically higher in the HIV-positive group compared to the HIV-negative group (n=35, 61% vs n=10, 38%; $X_2 = 7.5$, df=1, N=83, p=0.024).

No risk factors for mortality were identified in patients with CA-MRSA. The risk factors associated with mortality that were found in the HA-MRSA group were ICU admission (n=19, 46%), recent surgery (n=18, 44%), intubation (n=15, 37%), multi-organ dysfunction (n=9, 22%), and pyogenic complications (n=6, 15%). In the HCA-MRSA group the risk factors for mortality were inotropic support (n=2, 17%), multi-organ dysfunction (n=2, 17%) and pyogenic complications (n=2, 17%). The overall regression model was not significant (

F(6.93)=1.95, p=0.08, R_2 =0.11). Taken as a set the predictors in the three groups and the number of days from culture to outcome account for 11% of the variance in outcome. There was no statistical difference between ICU admission, inotropic support, intubation, recent surgery and pyogenic complications and the outcome, but there was a significant negative correlation between patients with multi-organ dysfunction and the outcome (R= -0.3, p <0.05).

HIV infection and MRSA

A total of 69% (n=57) of the tested study population were HIV-positive, with 17% (n=17/100) having an unknown HIV result. There was a statistically higher number of HIV-positive patients with known results between the three groups, with 63% (n=39) in HA-MRSA, 87% (n=13) in HCA-MRSA and 100% (n=5) in CA-MRSA. Thirty-four (60%) were on antiretroviral therapy, but only 16 (37%) of patients with a known viral load were virologically suppressed on treatment. There was no statistical difference in the CD4 count between the three groups, this being 125 cells/ul (IQR 179) in HA-MRSA, 27 cells/ul (IQR 49) in HCA-MRSA and 26 cells/ul (IQR 133) in CA-MRSA (H(2)=5.67, p=0.059).

HIV positive vs HIV negative patients

A total of 83 patients (83%) was tested for HIV. There was 57 HIV positive patients and 26 HIV negative patients in the study. The median age in the HIV positive group was 38 years (IQR 12; 20 – 59) and 32 (IQR 30; 23 – 82) years in HIV negative group (p value 0.341. There was a male predominance in both groups of 54% (n=31) and 59% (n=18) no statistical significance. There was no statistical significant difference between the length of hospital stay 24 days (IQR 26; 1 – 100) in HIV positive patients and 28 days (IQR 24, 9 – 85) in HIV

negative patients (p value 0.09). HIV negative patients were statistically more likely to be admitted to ICU than HIV positive patients 54 % (n=14) and 28% (n=16) respectively (p value 0.02). There was an increase in recent admissions in HIV positive patients with 53% (n=30) compared to 31% (n=8) in HIV negative patients (p value 0.06). There was a statistically significant difference in platelets between HIV positive and HIV negative patients 184 (IQR 234; 4 – 578) and 314 (IQR 265; 72 – 1327) respectively (p value 0.004). HIV patients had a significantly higher SOFA score at the time of culture of 5 (IQR 3; 0 -16) compared to 2 (IQR 2,5; 0 – 11) in HIV negative patients (p value 0.001). The all-cause mortality was statistically higher in the HIV-positive group compared to the HIV-negative group (n=35, 61% vs n=10, 38%; X_2= 7.5, df=1, N=83, p=0.024).

Antimicrobial management

Eighty-seven patients were started on empiric antibiotics, all of those with HCA-MRSA and CA-MRSA and 96% (n=74) of those with HA-MRSA. The most common empiric antibiotic used for HA-MRSA was piperacillin/tazobactam (46%, n=31) and glycopeptide (21%, n=14). In HCA-MRSA the most common empiric antibiotic used was ceftriaxone (36%, n=5) and piperacillin/tazobactam (21%, n=3) in the HCA group. The only two empiric antibiotics used in CA-MRSA were co-amoxiclav (60%, n=3) and ceftriaxone (40%, n=2). The empiric use of glycopeptide was 21% (n=14) in the HA-MRSA group and 7% (n=1) in HCA-MRSA.

In HA-MRSA 75% (n=54/72) of patients were placed on either vancomycin/linezolid post culture result and one was already on it. Nineteen patients did not receive appropriate antibiotics post culture results: 18% (n=12/72) died before results available, 4% (n=3/72) were discharged before the result became available and 6% (n=4) were not treated for MRSA despite

blood culture. In CA-MRSA two patients (40%) were changed to glycopeptide post culture results, two patients' results were not available prior to outcome, and one was not treated for MRSA and was discharged. In HCA-MRSA 76% (n=13/17) were treated with a glycopeptide; four were not treated with appropriate antibiotics and three died before the result was available while one was not treated for MRSA.

Discussion

The prevalence of MRSA bacteraemia in Southern Africa is between 30% and 38% with a prevalence of 0.03–0.08 per 1000 admissions in Gauteng [6 - 8]. The prevalence of CA-MRSA in Southern Africa has been established as 7.8% [8]. The current study had small numbers, it showed that there is indeed a lower prevalence of CA-MRSA compared to HA-MRSA; this is comparable with results of similar studies carried out elsewhere in South Africa, sub-Saharan Africa and Europe [11]. The prevalence of CA-MRSA has overtaken HA-MRSA as a cause of bacteraemia, especially in the USA a prevalence of CA-MRSA up to 73.6% [12]. Although HA-MRSA has been shown to be more common in winter months and CA-MRSA to be more predominant in summer, in the current study there was no statistical significance between the seasonality and the source of MRSA (see Table 1) [13,14].

Interestingly, there seems to be a male predominance of MRSA, which is in keeping with international and local data[15 – 21]. In this cohort 55/100 (55%) of patients were male, with a comparable predominance across all three groups. One study specifically looking at gender in MRSA attributed the reason for this as multifactorial, one reason that was provided is that men probably have poorer hand hygiene habits than their female counterparts [22]. Gender-based personal hygiene habits were also shown to contribute to the nasal colonization and

infection rates. Sexual dimorphism may play a role with regard to oestrogen, and has been observed in infections related to cholera and *Escherichia coli*; oestrogen is able reduce secretions from intestinal cells infected by these organisms, but there are limited data on this with regard to MRSA [22].

The patients across all three groups and especially those in the HA-MRSA group were younger when compared to international studies. From international data patients tended to be over the age of 65 years, with CA-MRSA occurring in slightly younger patients[15 – 21, 23]. The median age in HA-MRSA was 38 years, and this was only slightly higher in the CA-MRSA and HCA-MRSA groups (45 and 47 years respectively). This was comparable to a study by Perovic *et al*. in 2017 showing that only 20.4% of HA-MRSA and 8% of CA-MRSA patients to be older than 60 years [8]. One explanation for this in our study could be that the majority of the HA-MRSA patients were trauma patients, who tend to be male and of a younger age[24]. A possible role of HIV infection and acquired immunosuppression could be a second reason for a younger patient population; it has been suggested that a younger age in HIV patients has been shown to increase the risk of developing MRSA [25].

Patients with HCA-MRSA had prior hospital exposure compared to those with HA-MRSA, of whom only 31% (n=24) had prior hospital exposure ($p<0.05$). This shows that patients with prior hospital exposure are at higher risk of developing MRSA. Other risk factors from literature that have been shown to increase risk for HCA-MRSA include diabetes, dialysis, patients colonized with MRSA and previous MRSA infection [23]. These factors were not shown to be associated with an increased risk of developing HCA-MRSA, except for recent hospital exposure (see Table 1).

The majority of HA-MRSA patients (55%) were surgical patients (42/77, $p<0.05$) and almost all CA-MRSA and HCA-MRSA (4/5, 80% and 18/18, 100% respectively, $p< 0.05$) were medical patients. It has been shown that MRSA increases the length of hospital stay compared to the sensitive strain; in this study the length of stay was significantly longer in the HA-MRSA group than the other two groups, but when looking at length of stay from culture to outcome there was no significant increase in hospital stay between the groups[26]. While this shows that MRSA infection increases the overall hospital stay, there is no statistically significant difference between the HA-MRSA, CA-MRSA and HCA-MRSA groups [26].

When looking at the Charlson co-morbidity index it was shown that HA-MRSA patients had a lower score than those with CA-MRSA and HCA-MRSA. A study done by Chen et. al attributed the high prevalence of HCA-MRSA bacteraemia to a high co-morbidity index (4.6 +- 2.8), in this study patients with HCA-MRSA and CA-MRSA also had a similar high Charlson co-morbidity index[21]. This score uses points assigned to specific conditions to prognose mortality. Therefore patients with HCA-MRSA and CA-MRSA had a higher score, and thus more co-morbidities and possibly a reason for increased mortality. The most common co-morbid condition that was found in all three groups were HIV, this reflects the high rate of HIV infection in our setting.

Of the HA-MRSA group the majority of patients were admitted secondary to trauma (40/77, 52%), especially burns (35/40, 88%); with these patients admitted to the adult burns ICU, they were at high risk of developing HA-MRSA. Tuberculosis was the second most common reason for admission (17%, 13/77) and was the most common admission diagnosis in HCA-MRSA and CA-MRSA (44% or 8/18 and 40 or 2/5 respectively). Tuberculosis was diagnosed in 23% of patients with MRSA – much higher than found in a similar South African study that found

2.5% of HA-MRSA cases to have tuberculosis [7]. All of these patients had sensitive tuberculosis, which may be due to the high prevalence of tuberculosis together with HIV which has been shown to be a risk factor for MRSA infection, and nasal carriage may be an additional risk factor for development of MRSA[27].

Presumptive sites of infection in HA-MRSA are more likely to be secondary to skin and soft-tissue infection or intravenous catheter-related (24% or 19 /77 and 21% or 17/77 respectively). This is different when compared to other studies, where pneumonia was the most common followed by skin and soft tissue and bones and joints[28]. Of these cases (67.5% or 52/77) only had a bacteraemia. Thirty-one cultures other than blood culture revealed MRSA; the most common were cultures from central lines and skin and soft tissue (21% or 16/77 and 14% or 11/77 respectively). This shows the importance of hospital hygiene (doctor to patient and patient to patient spread) as well as aseptic techniques when inserting central lines and urinary catheters.

Of the cohort 83 out of 100 patients were tested for HIV and 68% (n=57) tested positive. These patients were clinically advanced at stage 4 with a median CD4+ count of 58 cells/ul. Only 60% were on antiretrovirals and the majority were not virologically suppressed on this treatment.

The all-cause mortality was in keeping with international literature, where MRSA mortality varies between 23% and 58% [15 – 21, 23, 29]. In our cohort CA-MRSA and HCA-MRSA had a higher mortality than HA-MRSA, which may be due to patients not being suspected of having MRSA and therefore not being placed on the correct antibiotics. HA-MRSA patients were more likely to have been given a glycopeptide than those in the CA-MRSA and HCA-

MRSA groups. The mortality was lower in the ICU-admitted group (only cases of HA-MRSA), and such admission is therefore not considered a risk factor for mortality as in other South-African literature [30]. Finally, the mortality was also higher in the HIV-infected group, which may be regarded as a significant risk factor for mortality in our population. Possible reasons for the increased mortality in HIV positive group is that the patients had a notable higher SOFA score, the median CD4 count was very low in these patients (Clinically advanced), high rate of concomitant tuberculosis infection, there was a higher rate of recent admission in HIV positive patients and HIV positive patients were less likely to have been admitted to the ICU.

Conclusion

With the trend of increasing CA-MRSA, especially in the USA, it was important to identify the epidemiology of MRSA in Southern Africa and especially at CHBAH [12]. The rates of CA-MRSA were low in this study, this is in keeping with other studies carried out in Southern Africa, but due to the small numbers and the number of records missing this could be an underestimation of the true prevalence of CA-MRSA in our setting. The patients were predominantly young males; trauma and HIV maybe being contributing factors. HA-MRSA was predominantly found in surgical patients, predominantly burns, compared to HCA-MRSA and CA-MRSA which occurred mostly in medical patients. Previous hospital admission is an important risk factor for the presentation of MRSA infection on admission, and these patients should be empirically covered. The overall mortality is high compared to international data, and was statistically higher in the CA-MRSA and HCA-MRSA groups. Therefore studies looking specifically at HIV and MRSA should be undertaken as well as the development of local guidelines regarding the appropriate use of antibiotics in our setting with regards to suspected MRSA infections should be done.

Limitations

One of the major limitations was poor record-keeping by healthcare practitioners as well as the records department. Only 100 files out of 224 cases of MRSA bacteraemia had enough information to use, this may contribute to the low prevalence off CA-MRSA in this study. Genotyping was not performed on the CA-MRSA strains to see if they differed from the HA-MRSA strain, but there was no evidence to suggest prior hospital admission and most likely they were from the community. Not all bacteraemia had confirmatory cultures from other sites, but this is in keeping with international data where only a positive blood culture is often the case.

Figures and tables

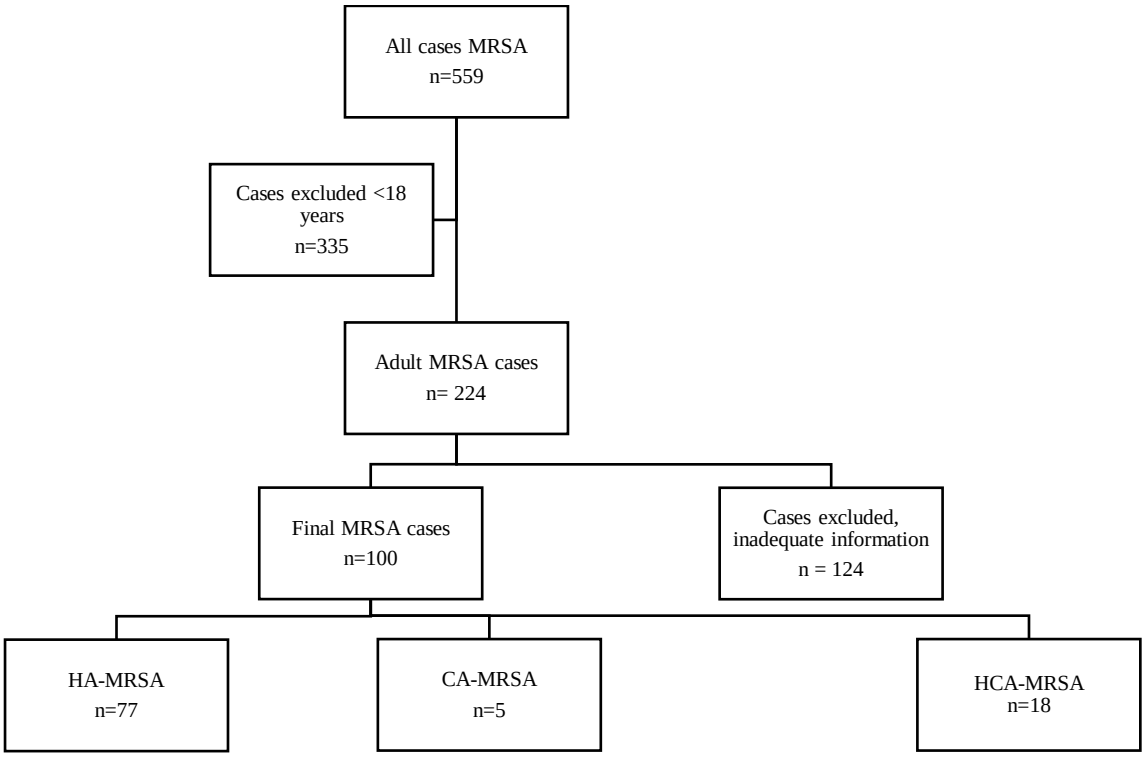

Figure 1:Profile of the study cohort..

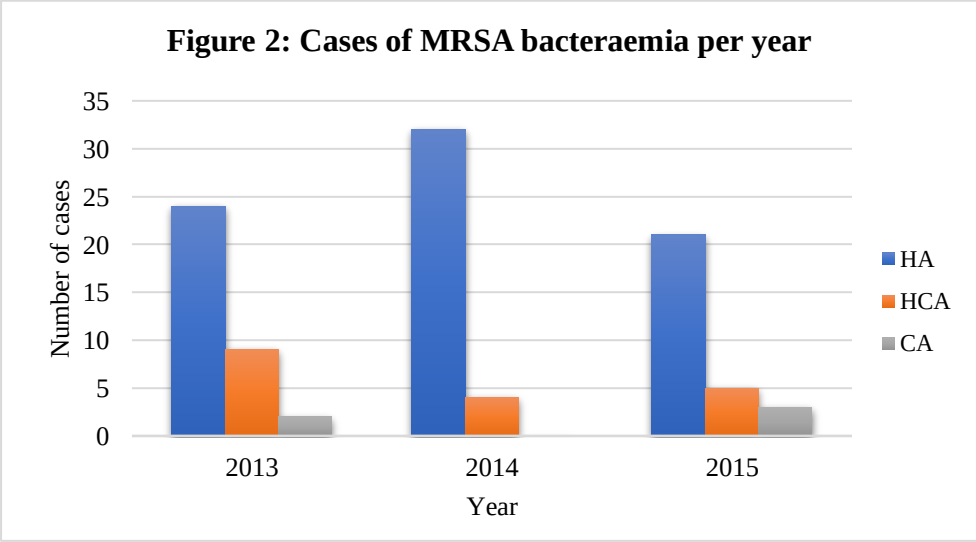

Table 1: Demographics, hospital stay of patients with MRSA bacteraemia at CHBAH (n=100)				
	HA n=77 (%)	**HCA n= 18 (%)**	**CA n= 5 (%)**	**p value**
Age (years) *	38 (IQR 21, 20–82)	47 (IQR 22, 20–80)	45 (IQR 17, 32–52)	0.184
Age groups				
18 - 29	20 (26)	3 (17)	0 (0)	
30 - 39	24 (31)	4 (22)	1 (20)	
40 - 49	13 (17)	3 (17)	3 (60)	
50 - 59	11 (14)	5 (28)	1 (20)	
60 - 69	5 (6)	1 (6)	0 (0)	
70 -79	3 (4)	1 (6)	0 (0)	
≥ 80	1 (1)	1 (6)	0 (0)	
Male gender	47 (61)	9 (50)	3 (60)	0.691
Race				
African	69 (89.6)	18 (100)	5 (100)	0.272
Caucasian	4 (5.2)	0 (0)	0 (0)	0.537
Coloured	2 (2.6)	0 (0)	0 (0)	0.737
Indian	2 (2.6)	0 (0)	0 (0)	0.737
Department				
Medical	34 (44)	18 (100)	4 (80)	<0.05
Surgical	42 (55)	0 (0)	1 (20)	<0.05
Psychiatry	1 (1)	0 (0)	0 (0)	0.86
Season				
Summer	22 (29)	4 (22)	1 (20)	0.807
Winter	25 (32)	4 (22)	3 (60)	0.273
Autumn	14 (18)	7 (39)	1 (20)	0.161
Spring	16 (21)	3 (17)	0 (0)	0.498
Hospital stay				
Length of stay (days) *	30 (IQR 26, 5–113)	8 (IQR 8, 1–33)	7 (IQR 20, 1–33)	<0.05
Days to culture (days) *	18 (IQR 17, 3–75)	0 (IQR 0, 0–1)	0 (IQR 0, 0–1)	<0.05
Days culture to outcome(days) *	13 (IQR 19, 0–74)	6 (IQR 20, 1–38)	7.5 (IQR 7.5, 1–33)	0.619
Recent admission	24 (31)	18 (100)	0 (0)	<0.05
Haemodialysis	9 (12)	3 (17)	0 (0)	0.589
Nursing home	0 (0)	1 (6)	0 (0)	0.100
Chemotherapy	2 (3)	1 (6)	0 (0)	0.749
Previous MRSA	2 (3)	2 (11)	0 (0)	0.226

ICU admission	39 (51)	0 (0)	0 (0)	<0.05

*** Expressed as median, CHBAH (Chris Hani Baragwanath Academic Hospital), MRSA (Methicillin resistant staphylococcus aureus), HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated), IQR (Inter-quartile range), ICU (Intensive care unit)**

Table 2: Clinical and laboratory features of patients with MRSA bacteraemia at CHBAH (n=100)				
	HA n=77	**HCA n= 18**	**CA n= 5**	**p value**
Clinical characteristic at time of blood culture				
Clinical features				
Temperature (∘C) *	37.5 (IQR 1.55, 36– 40); n=67	37 (IQR 1.36–40); n=17	37.4 (IQR 1.5, 36.2– 38.5); n=5	0.241
Heart rate (BPM) *	113 (IQR 23, 50– 153); n=69	106 (IQR 21, 51–146); n=18	120 (IQR 37, 97– 140); n=5	0.346
Respiratory rate (RPM) *	20 (IQR 4, 10–33); n=62	21 (IQR 4, 18–32); n=16	20 (IQR 0, 18–26); n=5	0.327
Systolic blood pressure (mmHg) *	120 (IQR 25, 59– 155); n=69	114 (IQR 29, 62–150); n=18	125 (IQR 27, 99–139); n=5	0.679
Mean arterial pressure (mmHg) *	86 (IQR 21, 45–120); n=69	81 (IQR 24, 47–118); n=18	92 (IQR 23, 68– 108); n=5	0.695
PaO_2 (mmHg) *	94 (IQR 60, 28– 317); n=8	44 (IQR 1, 43–44); n=2	95 (IQR 0, 95); n=1	0.493
P/F ratio *	233 (IQR 212.5, 70–1500); n=8	158 (IQR 47, 111–204); n=2	450 (IQR 0, 450); n=1	0.368
Glasgow Coma Scale *	15 (IQR 2, 2–15); n=65	15 (IQR 1, 8–15); n=18	15 (IQR 0, 15–15); n=5	0.217
Blood results				
White cell count ($x10^9$/l) *	12.75 (IQR 11.33, 1.3-40.94); n=76	7 (IQR 6, 2– 19); n=16	4.63 (IQR 1.62, 1.74 – 13.08) ; n=5	<0.05
Platelets ($x10^9$/l) *	226 (IQR 292, 4– 1327); n=75	206 (IQR 116, 51–486); n=16	206 (IQR 54, 145– 475); n=5	0.956
C-reactive protein (mg/l) *	200 (IQR 166, 6–787); n=66	164 (IQR 161, 18–335); n=16	108 (IQR 112, 14–181); n=4	0.071
Procalcitonin *	77,5 (IQR 125); n=26	-	-	N/A
Creatinine (umol/l) *	95 (IQR 183, 20–1146); n=75	134 (IQR 139, 35–944); n=16	67 (IQR 8, 48–143); n=5	0.381
Glomerular filtration rate(eGFR) *	96 (IQR 128, 4– 483); n=75	51 (IQR 80, 4–230); n=16	153 (IQR 21, 44– 165) n=5	0.256
Total bilirubin (umol/l) *	9 (IQR 13, 2–391); n=46	14 (IQR 15, 3–41); n=12	6 (IQR 5, 3–12); n=3	0.562
Albumin (g/l) *	23 (IQR 11, 9–39); n=70	25 (IQR 11, 14–41); n=14	30 (IQR 2, 28–32); n=2	0.419
International normalized ratio *	1.4 (IQR 1.7, 1.14–10); n=18	2 (IQR 0, 1– 2); n=5	1.23 (IQR 0, 1.23); n=1	0.532

Alanine aminotransferase (U/L) *	28 (IQR 27, 8–430); n=61	33 (IQR 65, 8–135); n=12	51 (IQR 29, 22–79); n=2	0.751
Aspartate aminotransferase (U/L) *	42 (IQR 46, 12–717); n=61	59 (IQR 46, 10–212); n=12	47 (IQR 9, 38–56); n=2	0.720
Alkaline phosphatase (U/L) *	117 (IQR 107, 32–1907); n-61	143 (IQR 47, 64–291); n=12	393 (IQR 156, 237– 549); n=2	0.119
Gamma-glutamyl-transferase (U/L) *	91 (IQR 164, 11– 2171); n=61	56 (IQR 107, 11–600); n=12	349 (IQR 1.5, 347– 350); n=2	0.157
SOFA Score *	4 (IQR 3, 0–16)	4 (IQR 3, 1– 9)	1 (IQR 1, 1–2)	<0.05

*** Expressed as median, CHBAH (Chris Hani Baragwanath Academic Hospital), MRSA (Methicillin resistant staphylococcus aureus), HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated), IQR (Inter-quartile range).**

Figure 3: Cases of MRSA bacteraemia in different age groups.

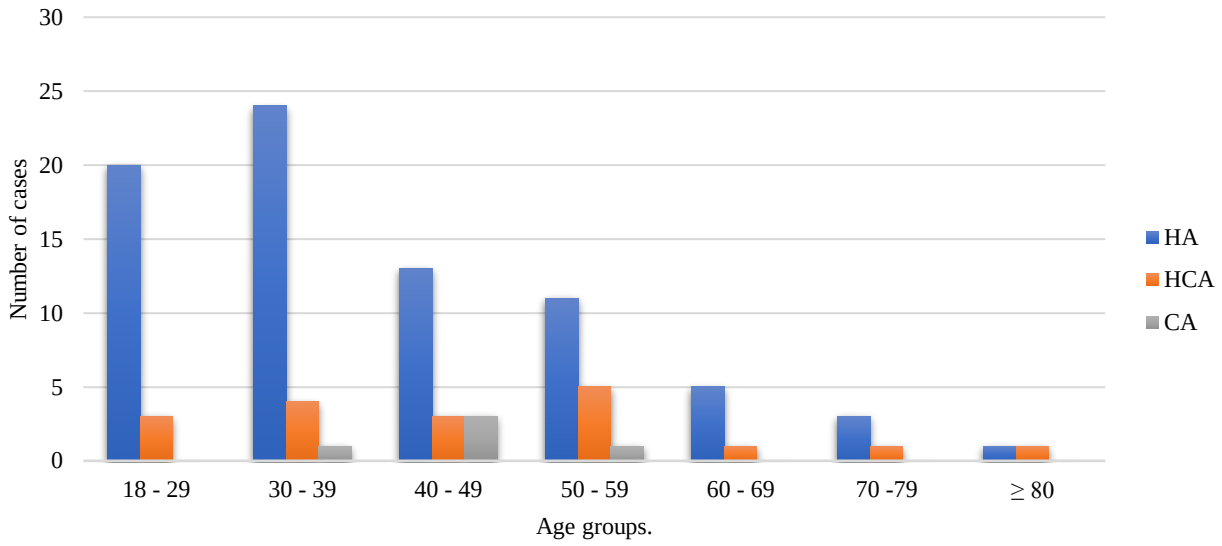

Figure 4: HA-MRSA Culture specimens.

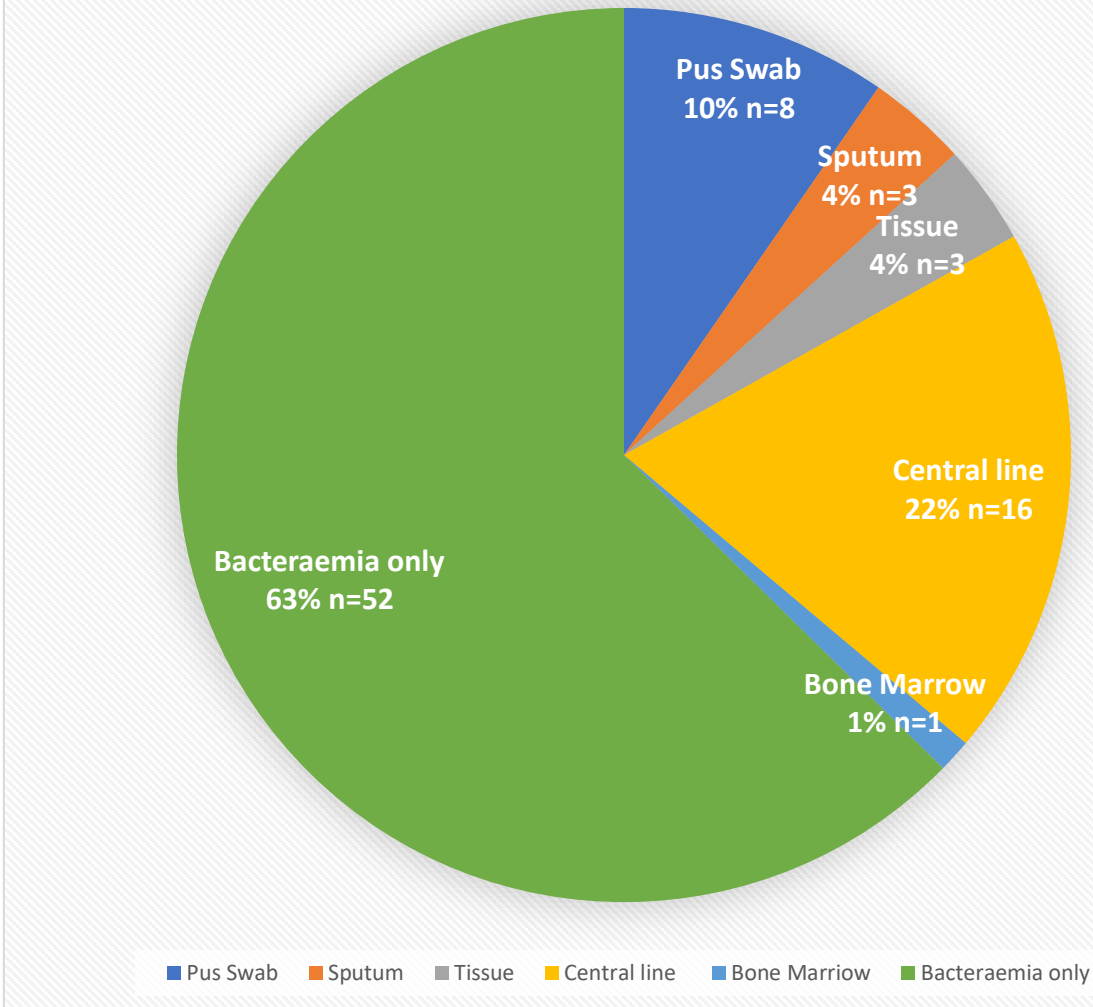

Table 3: Admission diagnosis for patients with MRSA bacteraemia.$					
	All patients (n =100) (%)	**HA (n=77) (%)**	**HCA (n=18) (%)**	**CA (n=5) (%)**	**P value**
Infections	57	35 (45.45)	18 (100)	4 (80)	
Tuberculosis	23	13 (16.88)	8 (44.44)	2 (40)	<0.05
Skin, bone and soft-tissue infections	11	10 (12,99)	1 (5.56)	0	0.479
Pneumonia	6	4 (5.19)	1 (5.56)	1 (20)	0.400
Meningitis	4	2 (2.60)	1 (5.56)	1 (20)	0.147
Clostridium Difficile	4	2 (2.60)	2 (11.11)	0	0.266
Urogenital infections	2	1 (1.30)	1 (5.56)	0	0.482
Acute gastroenteritis	2	2 (2.60)	0	0	0.737
Infective endocarditis	1	1 (1.30)	0	0	0.860
Pneumocystis pneumonia	1	0	1 (5.56)	0	0.100
Line sepsis	1	0	1 (5.56)	0	0.100
Sepsis	1	0	1 (5.56)	0	0.100
Cytomegalovirus retinitis	1	0	1 (5.56)	0	0.100
Trauma	40	40 (51.95)	0	0	
Burns	35	35 (45.45)	0	0	<0.05
Subdural haematoma	2	2 (2.60)	0	0	0.737
Stab to the heart	1	1 (1.30)	0	0	0.86
Neck of femur fracture	1	1 (1.30)	0	0	0.86
Gunshot abdomen	1	1 (1,30)	0	0	0.86
Other	34	23 (29.87)	9 (50)	3 (60)	

*****Other: Small bowel obstruction (HA: 3.90%). Drug eruption (HA:1,30% , HCA: 1,30% CA: 1,30%), Diabetic ketoacidosis (HCA: 16,67%), Iatrogenic Arteriovenous fistula (HA: 2,60% HCA 5,56%), Aplastic anaemia (HA: 1,30%, HCA: 5,60%), IgA dermatosis (HA 2,60%), Heart failure (HA 1,30%, HCA: 5,56%), Renal failure (HA: 1,30%, HCA 5,56%), Thrombotic thrombocytopenic microangiopathy (HA: 1,30%), Deep vein thrombosis (HA 1,30%), Anaemia (HA: 30%), Immune mediated thrombocytopenia (HA: 1,30%), HIV associated cholangiopathy (HA: 1,30%), Ascites (CA: 20%), Colorectal cancer (HA: 1,30%), Pancreatitis (HA: 1,30%), Panhypopituitarism (HA: 1,30%), Pulmonary embolism (HCA: 5,56%), Psychosis secondary to general medical condition (HA: 1,30%), Bronchiectasis (CA: 20%), Cauda equina syndrome (HA: 1,30%), Myelopathy (HA: 1,30%). □ Expressed as median. $ Some patients had more than one diagnosis on admission. CHBAH (Chris Hani Baragwanath Academic Hospital), MRSA (Methicillin resistant *Staphylococcus aureus*), HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated), IQR (Inter-quartile range).

Table 4: Most common presumed site of infection and other specimens that cultured MRSA in patients with MRSA bacteraemia.

	HA n=77(%)	HCA n=18(%)	CA n=5(%)	P value
Skin and soft tissue	19 (24.36)	1 (5.56)	1 (20)	0.200
Bone and joint	1 (1.28)	0	0	0.860
Catheter associated	17 (21.79)	2 (11.11)	0	0.305
Pneumonia	8 (10.26)	2 (11.11)	2 (40)	0.141
Endocarditis	1 (1.28)	0	0	0.860
Surgical wound	0	1 (5.56)	0	0.100
Central nervous system	0	1 (5.56)	0	0.100
Pus swab	8 (10.39)	2 (11.11)	0	0.743
Sputum	3 (3.90)	0	0	0.630
Tissue	3 (3.90)	0	0	0.630
Central line	16 (20.78)	0	0	0.580
Bone marrow	1 (1.30)	0	0	0.860
Bacteraemia only	52 (62.65)	16 (88.89)	5 (100)	0.070

CHBAH (Chris Hani Baragwanath Academic Hospital), MRSA (Methicillin resistant *Staphylococcus aureus*), HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated).

Table 5: Co-morbidities according to Charlson index in patients with MRSA bacteraemia.				
	HA n=77 (%)	**HCA n=18 (%)**	**CA n=5 (%)**	**P value**
AIDS	31 (40)	12 (67)	4 (80)	<0.05
Moderate or severe renal failure	27 (35)	8 (44)	0 (0)	0.183
Mild liver disease	20 (26)	3 (17)	1 (20)	0.691
Cerebrovascular disease	4 (5)	1 (6)	0 (0)	0.869
Diabetes mellitus with no target organ damage	2 (2,6)	3 (17)	0 (0)	0.041
Congestive heart failure	3 (4)	1(6)	0 (0)	0.850
Hemiplegia	3 (4)	1 (6)	0 (0)	0.850
Diabetes mellitus with target organ damage	2 (3)	2 (11)	0 (0)	0.226
Dementia	1 (1)	2 (11)	0 (0)	0.082
Any tumour	2 (3)	1 (6)	0 (0)	0.740
Peripheral vascular disease	2 (3)	0 (0)	0 (0)	0.737
Lymphoma	0 (0)	1 (6)	1 (20)	<0.05
Moderate or severe liver disease	1 (1)	1 (6)	0 (0)	0.483
Hypothyroidism	1 (1)	0 (0)	0 (0)	0.86
Atrial fibrillation	1 (1)	0 (0)	0 (0)	0.86

CHBAH (Chris Hani Baragwanath Academic Hospital), MRSA (Methicillin resistant *Staphylococcus aureus*), HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated), AIDS (Acquired immunodeficiency syndromes).

Table 6: Outcome in HA vs HCA vs CA MRSA bacteraemia patients.				
	HA n=77(%)	**HCA n=18(%)**	**CA n=5(%)**	**P value**
Outcome				
Discharged alive	35 (45)	6 (33)	2 (40)	0.64
Mortality ≤ 14 days	6 (8)	10 (56)	1 (20)	<0.05
Mortality ≤ 30 days	19 (25)	2 (11)	0 (0)	0.221
Mortality >30 days	16 (21)	0 (6)	0 (0)	0.058
Refused hospital treatment	1 (1)	0 (17)	1 (20)	0.012
All-cause mortality	41 (53)	12 (67)	2 (40)	0.463
HIV vs non-HIV mortality	**HIV- positive n=57**	**HIV- negative n=26**		
All-cause mortality	35 (61)	10 (38)		<0.05
ICU and HA-MRSA	**ICU n=39**	**No ICU n=38**		
All-cause mortality	19 (49)	22 (58)		<0.05

CHBAH (Chris Hani Baragwanath Academic Hospital), MRSA (Methicillin resistant *Staphylococcus aureus*), HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated), HIV (Human immunodeficiency virus), ICU (Intensive care unit).

Table 7: Risk factors for mortality before and after multivariate analysis.					
Risk factor for mortality	**HA n=41(%)**	**HCA n=12(%)**	**CA n=2(%)**	**p value**	**p value (Multivariate analysis)**
ICU admission	19 (46)	0 (0)	0	<0.001	0.17
Inotropic support	7 (17)	2 (17)	0	<0.001	0.44
Intubation	15 (37)	1 (8)	0	<0.001	0.78
Multi-organ dysfunction	9 (22)	2 (17)	0	<0.001	0.04
Pyogenic complications	6 (15)	2 (17)	0	<0.001	0.10
Recent surgery	18 (44)	1 (8)	0	<0.001	0.82

HA (Hospital-acquired), CA (Community-acquired), HCA (Health-care associated), ICU (Intensive care unit).

References

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. Lancet. 2006; 368(9538):874–85.
2. Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol. 2008 Dec; 8(6):747–63.
3. Hübner C, Hübner NO, Hopert K, Maletzki S, Flessa S. Analysis of MRSA-attributed costs of hospitalized patients in Germany. Eur J Clin Microbiol Infect Dis. 2014; 33(10):1817–22.
4. Lowy F. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003; 111(9):1265–73.
5. David MZ, Daum RS, Bayer AS, Chambers HF, Fowler VG, Miller LG, et al. Staphylococcus aureus bacteremia at 5 US Academic Medical Centers, 2008-2011: Significant geographic variation in community-onset infections. Clin Infect Dis. 2014; 59(6):798–807.
6. Fortuin-de Smidt MC, Singh-Moodley A, Badat R, Quan V, Kularatne R, Nana T, et al. Staphylococcus aureus bacteraemia in Gauteng academic hospitals, South Africa. Int J Infect Dis. 2015; 30:41–8.
7. Shuping LL, Kuonza L, Musekiwa A, Iyaloo S, Perovic O. Hospital-associated methicillin-resistant Staphylococcus aureus: A cross-sectional analysis of risk factors in South African tertiary public hospitals. PLoS One. 2017; 12(11):1–14.
8. Perovic O, Singh-Moodley A, Govender NP, Kularatne R, Whitelaw A, Chibabhai V, et al. A small proportion of community-associated methicillin-resistant Staphylococcus

aureus bacteraemia, compared to healthcare-associated cases, in two South African provinces. Eur J Clin Microbiol Infect Dis. 2017; 36(12):2519–32.

9. Charlson ME, Pompei P, Ales KL, Mackenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J Chron Dis. 1987; 40(5):373–83.

10. Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM et. al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine.1998; 26(11): 1793 – 800.

11. Köck R, Becker K, Cookson B, van Gemert-Pijnem JE, Harbarth S, Kluytmans JAJW, et al. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 2010; 15(41):1–9.

12. David MZ, Daum RS, Bayer AS, Chambers HF, Fowler VG, Miller LG, et al. Staphylococcus aureus bacteremia at 5 US Academic Medical Centers, 2008-2011: Significant geographic variation in community-onset infections. Clin Infect Dis. 2014; 59(6):798–807.

13. Leekha S, Diekema DJ, Perencevich EN. Seasonality of staphylococcal infections. Clin Microbiol Infect. 2012; 18(10):927–33.

14. Klein EY, Sun L, Smith DL, Laxminarayan R. The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: A national observational study. Am J Epidemiol. 2013; 177(7):666–74.

15 Gasch O, Ayats J, Angeles Dominguez M, Tubau F, Liñares J, Peña C, et al. Epidemiology of Methicillin-Resistant Staphylococcus aureus (MRSA) Bloodstream

Infection: Secular Trends Over 19 Years at a University Hospital. Medicine (Baltimore). 2011; 90(5):319–27.

16 Wyllie DH, Peto TE a, Crook D. MRSA bacteraemia in patients on arrival in hospital: a cohort study in Oxfordshire 1997-2003. BMJ. 2005; 331:992.

17 Allard C, Carignan A, Bergevin M, Boulais I, Tremblay V, Robichaud P, et al. Secular changes in incidence and mortality associated with Staphylococcus aureus bacteraemia in Quebec, Canada, 1991–2005. Clin Microbiol Infect. 2008; 14:421–8.

18 Kempker RR, Farley MM, Ladson JL, Satola S, Ray SM. Association of methicillin-resistant Staphylococcus aureus (MRSA) USA300 genotype with mortality in MRSA bacteremia. J Infect. 2010; 61(5):372–81.

19 Rahikka P, Syrjanen J, Vuento R, Laine J, Huttunen R. Meticillin-resistant Staphylococcus aureus (MRSA) bacteraemia in Tampere University Hospital: a case-control study, Finland October 2002 to January 2010. Eurosurveillance. 2011; 16(35):17–21.

20 Van Cleef BAGL, Kluytmans JAJW, van Benthem BHB, Haenen A, Monen J, Daniels-Haardt I, et al. Cross border comparison of MRSA bacteraemia between The Netherlands and North Rhine-Westphalia (Germany): a cross-sectional study. PLoS One. 2012; 7(8):e42787.

21 Chen L-Y, Chen L-K, Chang C-W, Kuo S-C, Li L-F, Chan Y-J, et al. Treatment of community-onset methicillin-resistant Staphylococcus aureus (MRSA) bacteremia: a hospital-based study. Arch Gerontol Geriatr. 2012; 55(1):152–6.

22. Humphreys H, Fitzpatick F, Harvey BJ. Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant staphylococcus aureus: Are they real, do they matter and why? Clin Infect Dis. 2015; 61(11):1708–14.

23. Pearson JC, Christiansen KJ, Coombs GW, Murray RJ. Community-associated versus healthcare-associated methicillin-resistant Staphylococcus aureus bacteraemia: A 10-year retrospective review. Eur J Clin Microbiol Infect Dis. 2009; 28(4):353–61.

24. Byarugaba J, Kielkowski D. Reflections on trauma and violence-related deaths in Soweto, July 1990 – June 1991. SAMJ. 1994; 84(9): 600 – 614.

25. Shadyab AH, Crum-Cianflone NF. Methicillin-resistant Staphylococcus aureus (MRSA) infections among HIV-infected persons in the era of highly active antiretroviral therapy: A review of the literature. HIV Med. 2012; 13(6):319–32. Robinson JO,

26. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol. 2005; 26(2):166–74.

27. Heysell S, Shenoi S, Catterick K, Thomas T, Friedland G. Prevalnce of methicillin-resistant *Staphylococcus aureus* nasal carriage among hospitilized patients with tuberculosis in rural KwaZulu-Natal. SAMJ. 2011; 101(5): 332 – 334.

28. Kallen AJ, Mu Y, Bulens S, Reingold A, Petit S, Gershman K, et al. Health care-associated invasive MRSA infections, 2005-2008. JAMA. [Internet]. 2010; 304(6):641–8.

29. Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA). Curr Opin Microbiol. 2012; 15(5):588–95.

30. Perovic O, Koornhof H, Black V, Moodley I, Duse A, Galpin J. Staphylococcus aureus bacteraemia at two academic hospitals in Johannesburg. S Afr Med J. 2006; 96(8):714–7.

Chapter 3: Appendix

i. Data collection sheet

DATA COLLECTION FORM:
METHICILLIN RESISTANT STAPHYLOCOCCUS AURES BACTERAEMIA IN ADULTS AT CHRIS HANI BARAGWANATH HOSPITAL

1. **Form:** ☐ **Patient identifier no:** ☐

2. **Demographics:**

   Age: ☐ Gender: Male ☐ Female ☐

   Race: African ☐ Caucasian ☐ Indian ☐ Coloured ☐

3. **Department:**

   Medical ☐ Surgical ☐ Obstetrics and Gynaecology ☐ Psychiatry ☐

4. **Hospital stay and history:**

   1. Date of admission: ☐

2. Outcome: Date

   Discharged Alive

   Mortality ≤ 14 Days

   Mortality ≤ 30 Days

   Mortality> 30 Days

3. Length of hospital stay in days:

4. Admission to ICU: Yes No

5. Recent hospital admission for more than two days in the last 90 days: Yes No

6. Haemodialysis: Yes No

7. Nursing Home: Yes No

8. Intravenous chemotherapy: Yes No

9. Previous MRSA: Yes No

10. Admission diagnosis:

5. Blood Culture:

Date	HA/CA	Poly	Isolate 1	Sensitivity	MIC	Isolate 2	Sensitivity	Isolate 3	Sensitivity	Isolate 4	Sensitivity

6. **Site of infection**:

Culture:

7. Laboratory values:

	Admission	Culture
WCC		
Platelets		
CRP		
PCT		
Creatinine		
eGFR		
Bilirubin		
Albumin		
INR		
ALT/AST		

ALP/GGT		

8. Clinical:

	Admission	Culture
Temperature		
Heart rate		
Respiratory rate		
Systolic blood pressure		
Mean arterial pressure		
PaO2		
Pa02/FIO2		
Glasgow coma scale		
Urine output		
SOFA		

9. **Co-Morbidities:** **Charlson Score**:

1	Myocardial infarction Yes ▬	Connective tissue disease Yes ▬
	Congestive Heart Failure Yes▬	Peripheral vascular disease Yes ▬
	Cerebrovascular disease Yes ▬	Dementia Yes ▬
	Chronic pulmonary disease Yes ▬	Ulcer disease Yes ▬
	Mild Liver Disease Yes ▬	Diabetes Mellitus Yes ▬

2	Hemiplegia Yes ___	Moderate or severe renal failure Yes ___
	Diabetes with end organ damage Yes ___	Any Tumour Yes ___
	Leukaemia Yes ___	Lymphoma Yes ___
3	Moderate or severe liver disease Yes ___	
6	AIDS Yes ___	Metastatic malignancy Yes ___
	Other:	

10. Risk factors mortality:

ICU Yes ___ No ___

Inotrope support Yes ___ No ___

Intubation Yes ___ No ___

Multi organ dysfunction Yes ___ No ___

Pyogenic complications Yes ___ No ___

Recent Surgery Yes ___ No ___

11. HIV:

Positive ___ Negative ___ Unknown ___ CD4+ VL suppressed Yes ___ No___

Unknown ___ ART Yes___ No___ Unknown ___

WHO ☐ Stage ☐

12. Antibiotics

Pre-antibiotic cultures Yes ___ No ___

Empiric antibiotic Yes ▁ No ▁ Choice:

Post-culture antibiotic choice:

Appropriate Yes ▁ No ▁

Duration of treatment: ≤ 7 Days ▁ ≤ 14 Days ▁ ≤ 21 Days ▁ N/A ▁

ii. Ethics Clearance Certificate

R14/49 Dr Guillaume Erich Holz

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160925

NAME: (Principal Investigator)	Dr Guillaume Erich Holz
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Methicillin Resistant Staphylococcus Aureus Bacteraemia in Adults at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	30/09/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof C.N Menezes and Dr F Sahid
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	18/11/2016

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature

18/11/2016
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

iii. Protocol amendment request to include 2013 and 2014 in study

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

17 July 2017

Dr Guillaume Erich Holz
Internal Medicine
Chris Hani Baragwanath Academic Hospital

Sent by email to: geholz@yahoo.com

Dear Dr Holz

Re: **Protocol Ref no: M160925**
Protocol Title: Methicillin Resistant Staphylococcus Aureus Bacteraemia in Adults at Chris Hani Baragwanath Academic Hospital
Principal Investigator: Dr Guillaume Erich Holz
Protocol Amendment: Request to Include Previous years

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved the protocol amendment on the abovementioned protocol, as detailed in your letter dated 12 June 2017.

Thank you for keeping us informed and updated.

Yours Sincerely,

L. moeng

Mr Lebohang Moeng
Administrative Assistant
Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
17 JUL 2017
RESEARCH OFFICE HREC (Medical)

Research Office Secretariat: Faculty of Health Sciences, Phillip Tobias Building, 3rd Floor, Office 302, Corner York Road and 29 Princess of Wales Terrace, Parktown, 2193 Private Bag 3, Wits 2050 I T+27 (0)11-717-1234/2656/2700/1252 E: Lebo.Moeng@wits.ac.za I Office E HREC-Medical.ResearchOffice@wits.ac.za IWebsite: www.wits.ac.za/research/about-our-research/ethics-and-research-integrity/

iv. NHLS and CHBAH clearance letter

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

01 November 2016

Applicant: Dr. Guillaume Erich Holz
Institution: University of the Witwatersrand
Department: Internal Medicine
Email: geholz@yahoo.com
Tel: 082 854 4265

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to support a research project titled "**Methicillin resistant staphylococcus aureus bacteraemia in adults at Chris Hani Baragwanath Hospital**" using data from the NHLS CDW database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available without patient names to you to conduct the proposed study as outlined in the submitted application.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research. Any data related queries may be directed to Sue Candy, manager NHLS Corporate Data Warehouse, Tel: (011) 386 6036. Email: sue.candy@nhls.ac.za.

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 11 Aug 2016

TITLE OF PROJECT: Methicillin resistant staphylococcus aureus bacteraemia in adults at Chris Hani Baragwanath Hospital

UNIVERSITY: Witwatersrand

Principal Investigator: GE Holz

Department: Internal Medicine

Supervisor (If relevant): C Menezes/ F Sahid

Permission Head Department (where research conducted): Yes

Date of start of proposed study: Aug 2016
Date of completion of data collection: Dec 2018

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Human Research Ethics Committee of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 11 August 2016

.....
Approved/Not Approved
Hospital Management
Date: 28/08/16

v. Proof of poster presentation

Certificate of Attendance

Guillaume Holz

For Presenting A Poster Presentation

Methicillin Resistant Stahphylococcus Aureus Bacteraemia in Adults at Chris Hani Baragwanath Academic Hospital

34 World Congress of Internal Medicine

Cape Town South Africa, 18-21 October 2018

Dr Adri Kok - Chairperson
PRESIDENT of Faculty of Consulting Physicians of South Africa
PRESIDENT-ELECT of the International Society of Internal Medicine

vi. Turn-it-in originality report

0701042p:Final_MMED_Submission_ready.docx

ORIGINALITY REPORT

22%	19%	19%	12%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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4	www.cambridge.org Internet Source	1%
5	Submitted to University of Witwatersrand Student Paper	1%
6	"Poster Sessions", Clinical Microbiology and Infection, 04/2012 Publication	<1%
7	tampub.uta.fi Internet Source	<1%
8	MJ Sucre. "Useful of implementation of neurally adjusted ventilatory assist in critically ill patients", Critical Care, 2010 Publication	<1%

vii. Additional changes made examiner 1

Recommendation 5:

Page 61, Paragraph 3, Line 1, Sentence 1: Added in the conclusion "The rates of CA-MRSA were low in this study, this is in keeping with other studies carried out in Southern Africa, but due to the small numbers and the number of records missing this could be an underestimation of the true prevalence of CA-MRSA in our setting."

Recommendation 6:

Page 62, paragraph 2, line 3, sentence 2: Added in limitation that the true prevalence of CA-MRSA may have been lower due to missing records.

Recommendation 8:

Extended literature review and manuscript single sentences removed and joined into paragraphs

- Page 12, Joined Paragraph 2,3,4 (Original page 10).
- Page 13, Joined paragraph 2 and 3(Original page 11).
- Page 13, Joined paragraph 1 (Original page 12 and paragraph 1 (Original page 11).
- Page 14, Joined paragraph 3,4 and 5 starting with paragraph (Original page 12
- Page 14, Joined paragraph 5(Original page 12) and Paragraph 1 (Original page 13).
- Page 14, Joined Paragraph 3 and 4 (Original page 13)
- Page 15 – 20 rewrote the epidemiology section, placed risk factors on its own.

- Page 20 paragraph 2: Joined paragraph 1, 2 (Original page 19) and paragraph 1 (Original page 20).
- Page 21, Paragraph 1: Joined paragraph 1,2 and 3 (Original page 21).
- Page 21, Paragraph 2: Joined paragraph 4 and 5(Original page 21) and paragraph 3 (Original Page 22)
- Page 22, Paragraph 2: Joined paragraph 2 and 5 (Original page 22).
- Page 24, Paragraph 3: Joined paragraph 1 and 2 (Original page 25).
- Page 25, Paragraph 1: Joined paragraph 3 and 4 (Original page 25).
- Page 25, Paragraph 3: Joined paragraph 3 and 4(Original page 26)
- Page 47, Paragraph 1: Joined paragraph 1,2 and 4 (Original page 43)
- Page 48, Paragraph 1: Joined paragraph 1 – 4 (Original page 44)
- Page 49, Paragraph 2: Joined Paragraph 2 and 3 (Original page 45)
- Page 51, Paragraph 2: Joined Paragraph 2 and (Original page 47)
- Page 52, Paragraph 1: Joined 2 – 4 (Original page 48)
- Page 52, Paragraph 3: Joined paragraph 2 and 3 (Original page 49)
- Page 54, Paragraph 2: Joined Paragraph 1 – 3 (Original page 51)
- Page 57, Paragraph 2: Joined paragraph 2 – 4 (Original page 53)
- Page 57, Paragraph 3: Joined paragraph 5 (Original page 53) and paragraph 1 and 2 (Original page 54).
- Page 59, Paragraph 2: Joined paragraph 3 and 4(Original page 55)
- Page 61, Paragraph 2: Joined paragraph 3 and 4 (original page 57)

Additional recommendations:

- Have acknowledged that it was a small sample size and that the loss of records may have been the cause for the small number of CA-MRSA found in the study.

- Plagiarism declaration was added page 5.

viii. Additional changes made examiner 2

General comment:

1. **The plagiarism report is not included.**

    - Added plagiarism declaration: Page 5.
    - Turn-it-in originality report: Page 83.

2. **Page numbers for the appendices listed in the contents are incorrect.**

    - Corrected page number for the list of contents. Page 6.

3. **List of Tables and Figures, correct headings for for Table 6 and Figure 1.**

    - Page 9, List of figures, Changed Figure 1 heading to "Profile of the study cohort."
    - Page 9, List of Tables, Changed Table 6 heading to "Outcome in HA vs HCA vs CA MRSA bacteraemia patients."
    - Page 63: Changed Figure 1 heading to "Profile of the study cohort."
    - Page 70: Changed Table 6 heading to "Outcome in HA vs HCA vs CA MRSA bacteraemia patients."

4. **HAART this term is no longer used.**

- Removed HAART and replaced with ART (Antiretroviral therapy)
    a. Page 10, Nomenclature, Line 2.
    b. Page 22 pg 1, line 9.
    c. Page 23 pg 1, line 1.
    d. Page 32, Line 2.
    e. Page 77, Data collection sheet, Point 11.

Specific comments:

Abstract:

1. Page 46, Methods: page 46 paragraph 2 line 4 expanded on method section including what statistics was used, "Descriptive statistics was used using the Pearson chi-squared test and a p value of less 0.05 with a confidence interval of 95% was used as statistically significant."
2. Page 46, Conclusions: Page 46 paragraph 4 line 2 Added limitation of the study due to small sample size "but may be underestimated due to the small sample size".
3. Page 46: Added abstract to 46 in front of introduction.

Introduction:

1. Page 12, paragraph 2, Line 4: Corrected the grammar.

2. Page 13, paragraph 1, Line 1: Added the prevalence of MRSA Bacteraemia from Southern-African studies into the introduction section.
3. Page 13, paragraph 2: Combined original paragraph 1 and 2.
4. Page 13, Moved paragraph 5 before paragraph 3 and 4.
5. Page 18, Paragraph 1, added Study on colonization of MRSA in Africa by Schaumburg.

Epidemiology:

1. Page 14 -20: Rewrote and reordered the epidemiology section to follow chronologically and regionally, made it more concise, moved the suggested paragraphs together. Also moved risk factors on its own.

Page 24, Paragraph 2 line 1.

- Corrected the grammar.

Page 24, Paragraph 3, Line 3.

- Added increase "in".

Page 24, Paragraph 3, Line 5.

- In Risk factor section removed the additional wording: those on, those with, cases, patients undergoing and those with.

Page 25: Paragraph 3 line 2.

- Added "days" to after 7

Page 27: Paragraph 2, line 2, Aims

- Removed "I aim to" → This study aims to.

Page 33, Paragraph 2.

- Added the factors involved in calculating the sample size required for this study.

Page 28, Data collection.

- Listed the inclusion and exclusion criteria clearly.

Page 29.

- Listed the data that was going to be collected and collaborated with data collection sheet.

Page 31, Paragraph 2.

- Discussed the value of using the Charlson co-morbidity index and added references for validation studies.

Page 32, Paragraph 2.

- Added and explanation for the use of the SOFA score with reference.

Page 32, Paragraph 3.

- Line 1, Added explanation on when the clinical and laboratory data were taken.
- Line 2, Explained how the blood cultures were taken.

Page 35, Limitations, Paragraph 2.

- Added limitation: “This study also includes mainly African black patients and may not be applicable to the rest of the South-African population in the health care sector.”

Page 47, Paragraph 3, Line 1.

- Added The prevalence of MRSA bacteraemia in Gauteng was established to be around 0.03 – 0.08 cases per 1000 admissions with two recent studies looking at five hospitals in Gauteng and the western cape establishing the rate of MRSA bacteraemia at 30% with only 7.9% of these cases being CA-MRSA.

Page 49, Paragraph 3.

- Line 4, Wrote HIV in full as Human-immunodeficiency virus.
- Line 10, Added referenced for Charlson index and SOFA Score

Results:

Page 50 Results:

- Thank you for your suggestion to present my data in Hazard Ratio with confidence intervals. Please note that limited statistics was done on this study as the sample size was do small in the corresponding three groups to run Hazard ratios as the results will not be significant as sample size is too small.

Page 52:, paragraph 4 .

- In regard to the CRP, this was regarding the blood cultures in which there was more than one isolate thus a polymicrobial blood culture. CRP was used to indicate if the patient truly was septic or likely a contaminate. Only 16 of these 27 polymicrobial blood cultures did not have MRSA confirmed on another culture and 14/16 had a raised CRP at the time of that blood culture. Therefore, the two I am referring to is not out of the 100 cases, but out of the 27 polymicrobial blood cultures. I have clarified this in the section.

Changed all results to one decimal point.

Page 56, paragraph 1

- Added differences between HIV positive and HIV negative patients.

Page 59, Paragraph 3 line 7

- Added the emphasis that HIV was the most common co-morbid condition in our setting.

Page 54, Paragraph 2, Line 5

- Added that > 30-day mortality almost reached statistical significance.

Page 61, Paragraph 2, Line 9

- There was a statistical significance in the outcome between HIV positive and HIV negative group. Gave an explanation of possible reasons for increased mortality in HIV positive group.

Discussion:

Added references:

Page 58:

- Paragraph 2, Line 2, Sentence 1: Added reference 15 – 21 for the age of MRSA.
- Paragraph 2, Line 5 Sentence 3 added reference 22.
- Paragraph 3, Line 3, Sentence 1 and Line 8, Sentence 2: added referenced 15 – 21 and 24.

Page 58:

- Paragraph 3, Line 8, Sentence 5: Added reference regarding age group of trauma in our setting.

Page 59:

- Paragraph 2, Line 1, Sentence 1, other studies did not specifically mention which department patients were admitted with HA-or CA-MRSA.
- Referenced paragraph 2, Line 6, sentence 2 as 26.

Page 59:

- Paragraph 3, Line 1, Sentence 1 added reference no 21 comparing to other studies using Charlson score.

Page 60:

- Paragraph 2, Line 10, Sentence 4: Added refence regarding MRSA nasal carriage in TB patients.
- Paragraph 3, Line 4, Sentence 2: Added reference regarding site of infection.

Page 61:

- Paragraph 2, Line 2, Sentence 1: Added reference regarding outcome compared to other studies.
- Paragraph 2, Line 8, Sentence 4: Added reference regarding risk factor of ICU in other south African studies.
- Paragraph 3, Line 2, Sentence 1: added reference regarding increase CA-MRSA USA.

Conclusion:

Page 62:

- Paragraph 1, Line 7: Added the need for local guidelines as well as the need for further studies looking at HIV and MRSA in our setting.

Tables:

- Added p values to tables.
    - Page 64, Table 1
    - Page 65, Table 2
    - Page 67, Table 3
    - Page 68, Table 4

    - Page 69, Table 5
    - Page 70, Table 6 and Table 7
- Removed all % from tables
    - Page 64, Table 1
    - Page 65, Table 2
    - Page 67, Table 3
    - Page 68, Table 4
    - Page 69, Table 5
    - Page 70, Table 6 and Table 7
- Mentioned lab and clinical results that was in table 61 instead of just saying lab and clinical data in methods.
- Page 65 Table 2: Added that the GFR is an estimated GFR.
- Page 65 Table 2: Added all n values for the clinical and laboratory data.
- Page 67, Table 3: Added in table that there was more than one diagnosis.
- Page 67, Table 3: Added the other diagnoses at bottom of table.