The prevalence of Methicillin-Resistant *Staphylococcus aureus* in blood product hampers at the South African National Blood Service

By

Varsha Seoraj
Student Number: 200303468

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College of Health Sciences

University of Kwa-Zulu Natal

Durban, South Africa

Supervisor: Professor A Chuturgoon

Co-Supervisors: Dr Karin van den Berg and Dr Ravesh Singh

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DECLARATION

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Signed: ______________________ Date: __________ Prof. A. Chuturgoon

Signed: ______________________ Date: __________ Dr. K. Van Den Berg

Signed: ______________________ Date: __________ Dr. R. Singh

DEDICATIONS

To my darling baby girl
Anamika Vidia Seoraj

For years I prayed and I waited
But the days kept flying by
I thought we'd never find our way
I thought it was just Dad and I
But then one day, the path was revealed
So many tears fell from my eyes
The love we have for you
No man can ever deny

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To my Family, no words can describe how grateful I am. Viren, for always being my biggest supporter. Viren, Mum and Dad, for holding my baby when I couldn’t.

To my unwavering, unfaltering, ever-present Saraswathi Ma, Thank You.

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA), are strains of the Gram-positive cocci known to cause various health conditions. Patients suffer from abscesses, skin infections and more severe conditions such as osteomyelitis and septicaemia. These bacteria are highly resistant to antibiotics and bacteria such as these are a great risk to the public, especially since *Staphylococcus aureus* is an opportunistic bacterium. In 2016, a donor unit received for the production of eye serum at South African National Blood Service (SANBS), when quality controlled, tested positive for MRSA. The bacterial contamination was traced to a staff member at the clinic where the blood was donated. Little research has been conducted to determine if MRSA is a problem and if it could negatively affect the blood supply.

Aim: The aim of this study was to determine whether blood services contribute to the spread of MRSA and other bacterial pathogens through the blood product hamper system.

Materials and Methods: A cross-sectional study to determine the prevalence of MRSA on 850 blood product hampers moving between SANBS inventory laboratories and blood banks, was conducted at SANBS between August 2020 and May 2021. Hampers were swabbed with a Sigma-Transwab containing liquid Amies transport medium for the detection of MRSA. The swabs were cultured onto CHROMagar MRSA where a rose or mauve coloured colony confirmed the presence of MRSA. Bacterial contaminants which were detected during the testing procedure were isolated, and loaded onto the Vitek 2 Compact for bacterial identification.

Results: A total of 696 hampers were processed as per the study protocol (81.9%). Out of the 850 hampers planned to be swabbed, 143 (16.8%) hampers were not swabbed as a result of staff not performing the procedure and swabs from 11 hampers were omitted (1.3%) as they did not comply too protocol requirements. Of the 696 hampers swabbed, MRSA was not detected (0%) however, bacterial growth other than MRSA was observed. The most common isolates detected were *Aerococcus viridans, Rothia dentocariosa*, followed by *Bacillis* spp as well as *Stenotrophomonas maltophilia.*

Conclusion:

The study findings have shown that an effective hamper cleaning system is needed to safeguard the integrity of our blood supply. The findings of this study should be taken into consideration throughout all provinces at SANBS, for the consistent and regular cleaning of hampers, which carry blood and blood products.

PREFACE

The Biomedical Research Ethics Committee (BREC), University of Kwa-Zulu Natal (Ethics Clearance Certificate No: BREC/00000743/2019) and the South African National Blood Service Human Research Ethics Committee (HREC) (Clearance Certificate No: 2019/0493), approved this study.

Table of Contents

LIST OF ABBREVIATIONS

MRSA	:	Methicillin resistant *Staphylococcus aureus*
MDRO	:	Multi-drug resistant organisms
VRE	:	Vancomycin-resistant enterococci
WHO	:	World Health Organization
SANBS	:	South African National Blood Service
CRF	:	Coagulase reacting factor
PRSA	:	Penicillin-resistant *Staphylococcus aureus*
PVL	:	Panton-Valentine leucocidin
MCS	:	Microscopy, culture and sensitivity
PCR	:	Polymerase chain reaction
PFGE	:	Pulsed-field gel electrophoresis
HA-MRSA	:	Hospital-acquired MRSA / Healthcare acquired-MRSA
CA-MRSA	:	Community acquired-MRSA
MRCoNS	:	Methicillin-resistant coagulase negative *Staphylococci*
IPC	:	Infection prevention and control
CHG	:	Chlorhexidine gluconate
URL	:	Uniform Resource Locators
ID-GN	:	Gram negative bacillus identification
ID-GP	:	Gram positive cocci identification
QC	:	Quality control
PPE	:	Personal protective equipment
ICU	:	Intensive care unit

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: INTRODUCTION

1.1 Bacteria

Bacteria are found everywhere and are essential to our existence. They contribute to the ecosystem, the production of medication, the functioning of the human body, however, they are responsible for disease. In the medical fraternity, bacteria are responsible for the mortality and morbidity of humans (Sharma *et al.*, 2021). Bacteria become pathogenic when they adhere, grow on and invade a living host (Chiller *et al.,* 2001). As bacteria became pathogenic, humans began using antibiotics, and the over consumption led to the bacteria becoming resistant to the drugs: multi-drug resistant organisms (Medina and Pieper, 2016).

1.2 Multi-drug resistant organisms

In addition to the general population of bacteria, we have the multi-drug resistant organisms (MDROs). These bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *P. aeruginosa* and vancomycin-resistant enterococci (VRE), are known to develop resistance to various antibiotics rapidly and have the ability to spread in conducive environments (Basak *et al.*, 2016). The impact on healthcare is devastating and so is the impact on the economy. Patients require specific antibiotics, their disease state is prolonged, and so is the hospital stay. The cost to the patient, is higher (Thaden *et al.*, 2017). One of the most significant MDROs and the reason for this study is MRSA. This highly resistant strain of gram-positive cocci was first identified in the 1960s (Jevons *et al*., 1963). In the 1940's penicillin was used as a highly effective treatment for *S. aureus* infections. However the continued used of this drug resulted in the bacteria rapidly developing resistance to the antibiotic (Brumfitt and Hamilton-Miller, 1989) and thus became known as penicillin-resistant *S. aureus* (PRSA). Not long after the antibiotic, methicillin was used to treat the PRSA until *S. aureus* developed resistance to this drug as well, leading to MRSA (Boyle-Vavra and Daum, 2007).

1.3 Methicillin-resistant *Staphylococcus aureus*

In particular, MRSA, are strains of the Gram-positive cocci known to cause various health conditions. Patients suffer from abscesses, skin infections and more severe conditions such as osteomyelitis and septicaemia. These bacteria are highly resistant to antibiotics and in addition, have the ability to produce a biofilm which allows the bacteria to survive and resist destruction by disinfectants (Russotto *et al*, 2017), allowing for survival on surfaces for hours, days, and even weeks and can eventually be transmitted to humans via touch. Bacteria such as these are a great risk to the public especially since *S. aureus* is an opportunistic bacterium and opportunistic bacteria are known to cause disease in immunosuppressed individuals (Fishman, 2013). The World Health Organization (WHO) in 2014 reported that MRSA was responsible for 80% in the African Region, 90% in the America Region, 60% in the European Region and 80% in the Western Pacific Region (*WHO, 2014)* . Globally MRSA is known to cause infections mainly in hospital wards, is tested for regularly, and prevented with the introduction of infection prevention control policies and procedures.

1.4 Methicillin-resistant *S. aureus* at the South African National Blood Service

1.4.1 Vein-to-vein chain

At SANBS, blood is collected from donors and processed and tested before being transfused to a patient. The SANBS vein to vein chain starts at the donor clinic, where blood is collected in a sterile blood collection bag, by trained nurses and phlebotomists (Navigation To A Safe Blood Transfusion - SANBS Nurses guide, 2014; *SANBS Immunohaematology training manual*, 2020). Within thirty minutes of collection, a pre-conditioned eutectic is attached to the blood unit, and placed in a validated multi-hamper for transport to the SANBS Processing laboratory (*SANBS Immunohaematology training manual*, 2020). Here, the blood is separated into the different components: red cells, platelets and plasma. Red cells and platelets are sent for quality control testing followed by storage in the inventory laboratory. The inventory laboratory will then issue blood to the blood banks depending on the usage in the area. When a patient is in need, the doctor will request units of blood or blood products from the blood banks, where the staff will test before issuing for transfusion. This is where the vein to vein chain ends (*SANBS Immunohaematology training manual*, 2020).

1.4.2 Transport of blood

Blood and blood products are transported on a daily basis to and from blood banks and processing laboratories (*SANBS Immunohaematology training manual*, 2020). Staff in the laboratories handle the blood bags even before the blood is collected, right up until the issue of the unit for the patient. Inventory staff pack the blood product hampers, which is then transported to the blood banks, where staff unpack the hampers.

1.4.3 SANBS eye serum

Using a messenger application on 22 February 2022, Dr Ute Jentsch confirmed that in 2016, a donor unit was received for the production of eye serum at SANBS. Eye serum is one of the blood products which SANBS prepares for patients suffering with dry eye syndrome. The serum obtained was sent to the quality department for routine bacterial and fungal testing, and came back positive for MRSA. The unit was discarded and an investigation carried out where the bacterial contamination was traced back to a staff member at the clinic where the blood was donated.

1.5 Rationale for the study

Blood services, even though they form part of the healthcare delivery system, have very little literature published in the detection of MRSA in their facilities. At SANBS a case of MRSA has been detected, however, little research has been conducted to determine if MRSA is a problem and if it could negatively affect the blood supply. The study will determine, at the ground level, if MRSA and/or other pathogenic bacteria are prevalent and if they will have a negative impact on the blood service.

1.6 Hypothesis

Null hypothesis: The proportion of MRSA in hampers from inventory laboratory are equal to the proportion of MRSA in hampers from blood bank laboratories

Alternate hypothesis: The proportion of MRSA in hampers form inventory laboratory is not equal to the proportion of MRSA in hampers from blood bank laboratories

1.7 Aim

The aim of this study was to determine whether blood services contribute to the spread of MRSA and other bacterial pathogens through the blood product hamper system.

1.8 Objectives

- To determine the presence of MRSA in the blood service’s hamper system
- To determine the presence of bacteria in the blood service’s hamper system
- To determine whether there is a difference in either MRSA or other bacterial contamination between the movement of hampers within SANBS laboratories

CHAPTER TWO: LITERATURE REVIEW

2.1 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) is a gram-positive coccus, discovered by Sir Alexander Ogston in 1880, when he observed micrococcus in abscesses filled with pus (Ogston, 1881). These bacteria are non-spore forming and facultative anaerobes which can either be catalase positive or negative, which under the microscope can be observed as grape-like clusters (Todd, 2014). It is the most virulent of the *Staphylococcus* species and is associated with health issues ranging from minor skin infections, to toxic shock syndrome (Boyle-Vavra and Daum, 2007), infective endocarditis and central nervous system infections (Ondusko and Nolt, 2018). In the figure below (Fig. 1.1) micrococci are shown as discovered by Sir Alexander Ogston.

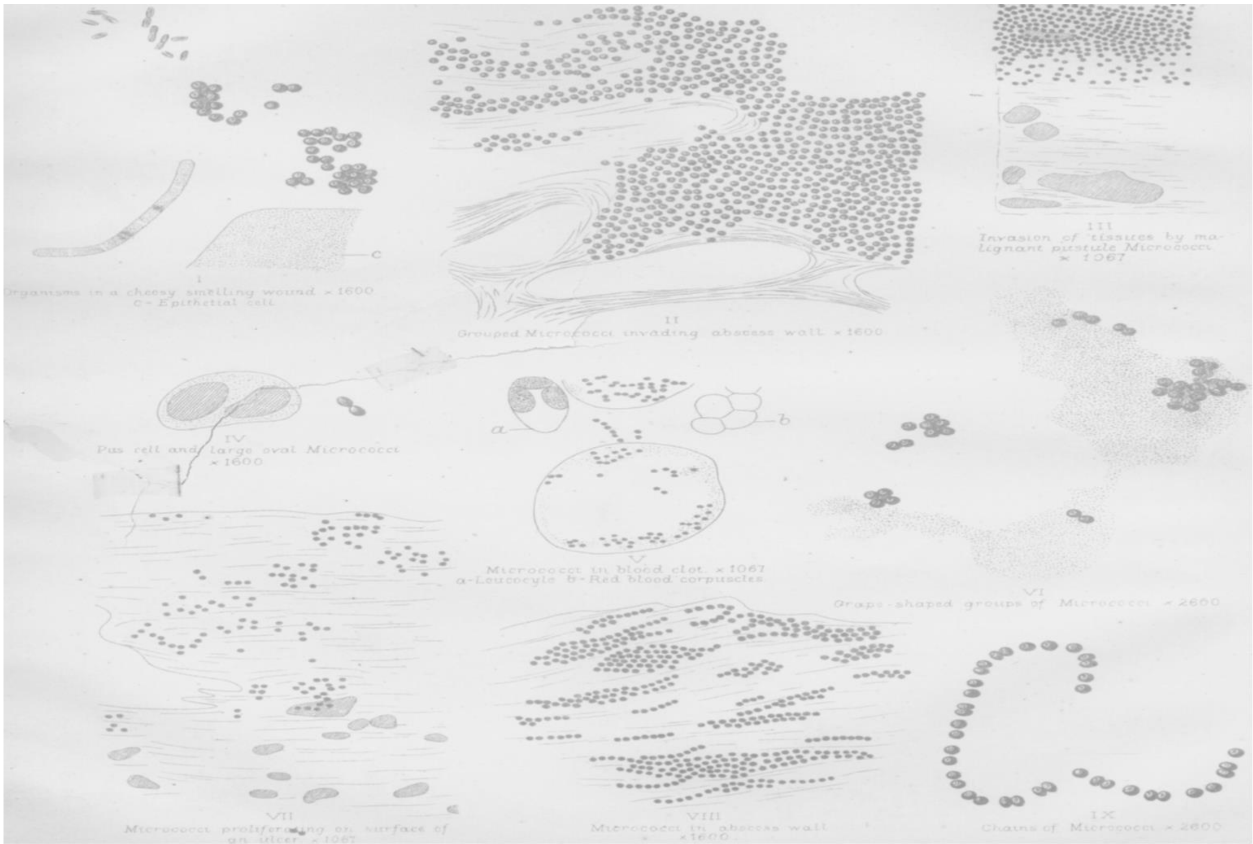

Figure 1.1: Group micrococci invading abscess wall (Adapted from Ogston, 1881)

These micrococci were observed as three or four cocci clustered together or sometimes as chains, and at times larger clusters and chains observed (Ogston, 1881).

Staphylococcus aureus has the added ability to produce biofilm. Biofilm occurs when *S. aureus* produces free coagulase. This coagulase activates a coagulase reacting factor (CRF) to form a complex called coagulase-CRF complex. This complex then binds with fibrinogen and forms a fibrin clot which results in the formation of the biofilm (Fey and Olson, 2010). Fig. 1.2 below shows the life cycle of biofilm.

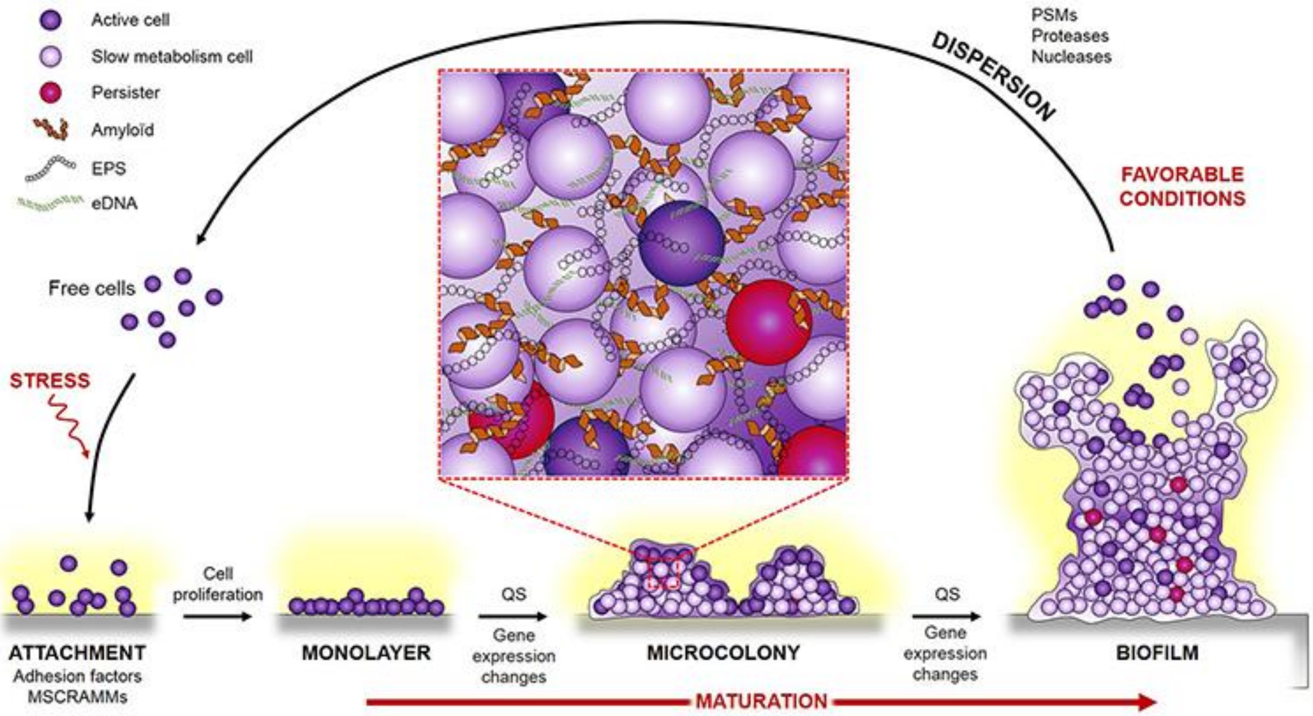

Figure 1.2: Biofilm Life Cycle (Reffuveille *et al*, 2016)

Biofilm protects and allows survival of the bacteria to avoid destruction by antibiotics and disinfectants.

Coagulase-negative staphylococci (CNS), on the other hand, are made up of different species of staphylococcus such as *Staphylococcus epidermidis* (Martins and Cunha, 2007) and have been regarded as non-pathogenic (Huebner and Goldman, 1993). Most times CNS is acquired during a hospital stay (Huebner and Goldman, 1993) and sometimes cause infection in immunocompromised individuals especially those connected to a catheter (Martins and Cunha, 2007).

In the 1940's benzylpenicillin, commonly known as penicillin, was used as a highly effective treatment for *S. aureus* infections. This was short-lived as the continued use of this drug resulted in the bacteria rapidly developing resistance by producing β-lactamase (Brumfitt and Hamilton-Miller, 1989), and thus became known as penicillin-resistant *S.*

aureus (PRSA). Thereafter, methicillin was the drug used to treat the PRSA for many years, until *S. aureus* developed resistance to this drug as well, leading to methicillin-resistant *Staphylococcus aureus* (MRSA*)* (Boyle-Vavra and Daum, 2007).

2.2 Methicillin-resistant *Staphylococcus aureus*: the Superbug

The highly resistant strains of gram-positive cocci was first identified in the 1960s (Jevons *et al*., 1963). Penicillin resistance of *S. aureus* is mainly due to the production of β-lactamase, whilst the methicillin resistance of *S. aureus* is a result of gaining genes which allow for the encoding of penicillin-binding proteins (Moellering, 2012). This penicillin-binding protein has a lessened affinity for β-lactams and is encoded by the *mecA* gene. (Moellering, 2012). The MRSA develops when methicillin-susceptible *Staphylococcus aureus* (MSSA) obtains the *mecA* gene, which is transported on a staphylococcal chromosome cassette *mecA* (SCC*mecA*) (Moellering, 2012). However, this *mecA* gene may not necessarily have originated from *S. aureus*, but rather from an animal species *S. sciuri* (Antignac and Tomasz, 2009). The effect of the *mecA* gene on biofilm production is of great importance. This gene increases the biofilm production for MRSA by inactivating a gene called the accessory gene regulator (agr) (Kirmusaoglu 2017 p. 25). .

Methicillin resistant *Staphylococcus aureus* is associated with a number of *S. aureus* lineages and clones. According to Enright *et al*. (2002), upon their analysis of 912 MRSA and MSSA isolates, they were able to identify 11 major MRSA clones (Enright *et al*, 2002). Moodley *et al*. (2010) found five major MRSA lineages in South Africa using various testing methods: pulsed-field gel electrophoresis (PFGE), spa typing, SCCmec typing, multilocus sequence typing (MLST) and detection of Panton- Valentine leukocidin (PVL) by PCR Moodley *et al*., 2010). A total of 320 MRSA isolates were cultured with 187 obtained from males ranging from newborn to 91 years old, 119 females ranging from newborn to 87 years old and 14 persons of unknown gender (Moodley *et al*., 2010). The various strains were taken from various sources such as bacteraemia's, skin and soft tissue infections, cerebrospinal fluid, urine and catheter tip (Moodley *et al*., 2010). The major lineages detected included: Type A, D, T and F (Moodley *et al.*, 2010). In addition, the study resulted in the detection of Type K; the most widespread clone. Type D was not seen in the Eastern and Western Cape and Type F was not detected in Limpopo, Gauteng and the North West Province (Moodley *et al.*, 2010). Durban in KwaZulu-Natal (KZN) (South Africa) was found to be positive for four of the five clones detected nationally, and Pretoria was positive for three of the clones (Moodley *et al.*, 2010). A study conducted in 14 KZN provincial hospitals, analysed 223 isolates obtained from wound samples, sputum, otitis media and blood samples (Shittu and Lin, 2006). The study, aimed at isolating, , characterizing and determining the susceptibility of *S. aureus,* especially MRSA, found that the MRSA isolated was resistant to gentamicin, erythromycin, clindamycin, rifampicin and trimethoprim (Shittu and Lin, 2006).

In Portugal and Rome, sensitivity tests conducted found that MRSA was resistant to cefoxitin, ciprofloxacin, clindamycin, erythromycin, fusidic acid, penicillin, rifampin, and tetracycline (Conceição *et al*, 2017). Studies found that the only antibiotics which MRSA was sensitive to vancomycin and linezolid making this bacterium a superbug. Marais *et al. (2009)* conducted their study in South Africa to determine the susceptibility of MRSA isolates in the country. The 248 MRSA isolates were collected from various National

Health Laboratory Services (NHLS) and private laboratories. These isolates were tested against 17 antibiotics was performed using the disc diffusion method (Marais et al, 2009). The researchers found the MRSA to be generally resistant to similar drugs: ciprofloxacin, erythromycin, gentamicin, tetracycline and trimethoprim, with no MRSA isolates resistant to vancomycin detected (Marais *et al.*, 2009).

Two types of MRSA have been described; hospital or healthcare acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA). The HA-MRSA was identified in hospitals as the cause of S. *aureus* infections globally, and remains a major source of nosocomial infections (Waters *et al.*, 2017). Individuals who are infected with MRSA, regardless whether they have few or no healthcare associated risk factors, are defined as having CA-MRSA (Boyle-Vavra and Daum, 2007).

Testing: Some of the methods used for MRSA detection include microscopy, culture and sensitivity (MCS) where the organism is cultured on specific chromogenic agar for a visual confirmation, polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (Moodley *et al.*, 2010). The widely used commercially available MRSA PCR kits being used currently are the DI-MRSA/GeneOhm manufactured by BD GeneOhm MRSA and GeneXpert MRSA (Cepheid), (McClure *et al*, 2020). The best specimens for MRSA detection was found to be nasal swabs, which had the highest detection rates compared to throat specimens (Young *et al.*, 2017). However, immune-compromised individuals, as well as healthy individuals have been found to carry the bacteria on their bodies *(Boyle-Vavra* and Daum, 2007). The bacteria can survive on inanimate objects for prolonged periods of time resulting in transmission from one person to another (Neely and Maley, 2000). Furthermore, Williams et al conducted a study where their results showed that various concentrations of MRSA on inanimate objects lead to various survival rates; i.e. the higher the concentration of MRSA, the longer the bacteria survives on the inanimate object (Williams and Davis, 2009). The results showed no live MRSA was detected from whole blood (50 CFUs/ml). However, MRSA in serum (2,000 CFUs/ ml) survived at various rates on the varying surfaces: 41 days on glass surfaces, 45 days on tiles, and a minimum of 60 days on a countertop (Williams and Davis, 2009). Furthermore, results showed that MRSA was present on 3 of the 15 stethoscopes tested from students involved in respiratory therapy and 2 of the 18 stethoscopes from nursing students (Williams and Davis, 2009).

2.3 Hospital and Community acquired MRSA

The most common site of MRSA colonization are nares of individuals (Conceição *et al*., 2017) and this contributes to nosocomial and community-acquired infections (Marais *et al.*, 2009). The resistance of methicillin by Staphylococcus aureus is a result if the acquisition of the *mec*A gene, which is carried by the element SCC*mec* (Asghar, 2014). There are five major types of SCC*mec* elements: hospital-acquired MRSA (HA-MRSA) strains carry SCC*mec* types I, II, or III, the majority, whilst community-acquired MRSA (CA-MRSA) strains carry SCC*mec* types IV or V. Waters *et al. (2017)* found that HA-MRSA had a high resistance to methicillin, but showed lower toxin production and lower virulence whilst, CA-MRSA showed resistance to lower doses of methicillin and was the cause of skin infection, pneumonia and even sepsis in healthy individuals (Waters *et al.*, 2017). By the 1990s, CA-MRSA led to more cases of fatal disease when compared to HA-MRSA (Boyle-Vavra and Daum, 2007). This confirms community spread of MRSA independent of exposure to a hospital setting. In South Africa, Perovic *et al.* (2017) conducted a study to compare the percentage of HA-MRSA and CA-MRSA in the Gauteng and Western Cape Provinces. What they found, in what may have been the biggest study in South Africa aiming at determining the prevalence of *S. aureus* in South Africa, was, 1914 cases of patients infected with *S. aureus*, 29.1% of which was classified as MRSA (Perovic *et al.*, 2017). Furthermore, 2.3% of the cases were classified as CA-MRSA, with the remaining 26.8% HA-MRSA (Perovic *et al.*, 2017). The researchers concluded that MRSA is common in South Africa, with HA-MRSA being the more prevalent type (Perovic *et al.*, 2017).

2.4 MRSA in healthcare settings

Routes of Transmission

In 2018, researchers produced an environmental panel in a Michigan nursing facility which included areas such as the bed rails, beds and TV remote controls for the detection of MRSA (Cassone *et al.*, 2018). The results showed that the TV remote, the bed rails, and the toilet seats were positive for MRSA and therefore suggested these environmental panels will allow for the identification of MRSA in the surrounding environment. A study conducted in 2008 looked at the aerial dispersion of MRSA. Air sampling was conducted using MRSA selective agar in hospital rooms occupied by MRSA infected or respiratory tract infected patients. Using antimicrobial resistance and PFGE, researchers found that in 21 of the 24 rooms, at least one of the isolates (environmental) found was identical to the isolate (clinical) found in another room (Gehanno *et al.,* 2009). The researchers concluded that viable MRSA could be shed into the air, and more research needed to be conducted to improve infection control (Gehanno *et al*., 2009). Supporting this conclusion, Kwok *et al. (2018)* conducted a study in another nursing facility where they found that MRSA were more evident during and after bed-making (Kwok *et al.*, 2018). Likewise, Zinderman *et al. (2004)* found that humidity played a significant role in the transmission of MRSA. In the warmer months individuals tend to have increased exposure of their skin, and thus increased exposure of and contact with other skin surfaces (Zinderman *et al.*, 2004).

A similar study conducted in Egypt with ambulance vehicles as a source of multidrug infections showed that *S. aureus* was detected and half of those isolates were MRSA (El-Mokhtar and Hetta, 2018). The bacteria was detected on the patient stretcher headboard, suction device, door grip, computer monitor, chairs, inner walls of the vehicles, beds, the sidebars of the carts as well as the cart handles (El-Mokhtar and Hetta, 2018). This indicates that transport vehicles are reservoirs for MRSA and allow transmission to occur.

2.5 MRSA in non-healthcare settings

Routes of Transmission

Methicillin resistant *Staphylococcus aureus* has been reported in multiple non-healthcare settings. These include prisons with an unexpectedly high MRSA carrier rate for a non-health-care facility (Center of Disease Control, 2001). It was noted that these individuals had constant contact with each other, which was probably the route for the spread of the MRSA. In addition to prison facilities, MRSA has been demonstrated in sports teams, the military and childcare settings. Football players from the St. Louis Rams team in Missouri, USA, tested positive for CA-MRSA, which was spread by one of the players to his fellow team members (Kazakova *et al.*, 2005). The MRSA infections were detected in military recruits but the infection with MRSA was not found to be from a single source as cases occurred throughout the training facility and the percentage of carriers was around 2% - 3% (Zinderman *et al.*, 2004). Another important factor to take into consideration is public transport systems. These transport systems are widely used around the world. Researchers Stepanović *et al. (2008)* studied the public transport system in Serbia. The researchers found that although MRSA was not detected in the transport vehicles, methicillin resistant coagulase negative *Staphylococc*i (CNS) was detected (Stepanović *et al.*, 2008). This bacteria is regarded as a reservoir for the *mecA* gene and can have the ability to change methicillin susceptible *Staphylococcus aureus* (MSSA) into MRSA due to the presence of the mecA gene (Li *et al.*, 2021). Making this another instance of impending MRSA contamination.

2.6 MRSA surveillance programmes

In 2018 the Michigan nursing facility looked at the patients as well as the environment as a source for patient colonization for MRSA (Cassone *et al.*, 2018). Further investigation using molecular typing using staphylococcal chromosome cassette (SCCmec) type, pulsed-field gel electrophoresis (PFGE) and the presence of the Panton-Valentine leukocidin (PVL) gene found that 54% of isolates were positive for HA-MRSA and 33% positive for strains considered to be CA-MRSA (Cassone *et al.*, 2018). Hospital-acquired MRSA (HA-MRSA) strains carry SCC*mec* types I, II, or III, the majority, whilst community-acquired MRSA (CA-MRSA) strains carry SCC*mec* types IV or V. However, Cassone *et al. (2018)* found surveillance programmes for MRSA were rarely conducted due to a lack of resources, patient denial, difficulties in patient schedules and sampling sites (Cassone *et al.*, 2018).

2.7 MRSA in blood transfusion services

Relating to blood transfusion, there is very little literature describing MRSA transmission between donors and blood transfusion service employees. A study conducted in Cleveland, Ohio identified a pooled platelet pack positive for MRSA after transfusion to an immune-compromised patient, which was believed to contribute to the death of that patient (Sapatnekar *et al.*, 2001). The platelets were transfused to the patient, and during the procedure, 10 ml of platelets were introduced into the patients' chest tube. At that time, the American Red Cross Blood Services routinely conducted surveillance cultures on all pooled platelets, which were 4-5 days old. Cultures obtained indicated that one donor platelet bag was positive for MRSA and led to the contamination of pooled platelets issued to the patient. Pleural fluid cultures were found to be positive for MRSA. It was concluded that the MRSA was detected due to the routine testing done by the Red Cross Blood Services (Sapatnekar *et al.*, 2001). Unfortunately, the study was unable to identify the source of the MRSA. The donor refused testing and the blood service staff were not tested for MRSA (Sapatnekar *et al.*, 2001).

In 2017, a study was conducted at The Northern Ireland Blood Transfusion Service after they obtained false negative results from the BacT/ALERT from bacterially contaminated platelet packs (Abela *et al.*, 2018). *Staphylococcus aureus* was isolated from the residue of three different platelet packs. Upon further investigation, two of the *S. aureus* strains found had a similar genetic makeup to the corresponding donors (Abela *et al.*, 2018). It was concluded that the cause of the false negative results was as a result of biofilm production (Abela *et al.*, 2018)..

At the South African National Blood Service (SANBS), whole blood is donated altruistically and the blood is separated into three different components: red cell concentrates, platelets and plasma (Medical Technician Blood Transfusion Training Manual, 2015). Bacterial contamination is limited during the donation process by cleaning the venipuncture site of the donor's arm with a 70% alcohol swab. Secondly, a plastic pouch connected to the main collection bag, receives the first draw of blood, thereby preventing any residual bacteria from the venipuncture site from entry into the main collection bag. Lastly, all units are closed systems, thus the external environment may not affect the collected blood. However, bacterial contamination may occur if the donor has a current bacteremia, if the venipuncture site was not cleaned sufficiently, if the blood collection bag is opened during the processing, or during pooling of platelets where a single unit is positive, such as the case reported by Sapatnekar *et al. (2001)*. Using a messenger application on 22 February 2022, Dr Ute Jentsch confirmed that in 2016, a donor's eye serum was quality controlled at SANBS and, upon testing, Methicillin resistant *Staphylococcus aureus* was detected. The environment and staff in both laboratory and clinic area were screened and the source was linked to a collection phlebotomist and in addition a significant proportion of the nurses were found to be carriers). All carriers were provided with appropriate treatment and retesting to confirm that the carrier state was eradicated.

2.8 Pathogenic bacteria

In a 2018 study, the detection of MRSA led to the detection of other bacteria including the vancomycin-resistant *Enterococcus* (Cassone *et al*, 2018)*, Escherichia coli, Vibrio cholera,* and *Mycobacterium tuberculosis* (Chowdhury *et al.*, 2016). El-Mokhtar *et al.* (2018) detected contaminants such as *Citrobacter* sp, *Proteus* sp, *Bacillus* sp, *Corynebactrium* sp and *Micrococcus* sp (El-Mokhtar and Hetta, 2018). A study conducted at SANBS between 2011 and 2016 looked at the presence of bacteria in apheresis platelets. Apheresis platelets are single donor units and are not often requested by doctors. The most common bacteria detected included coagulase negative Staphylococci*, Bacillus* sp*, Micrococcus* sp and streptococci (Jentsch and Swanevelder, 2019)*.* The pathogenic bacteria detected included *Acinetobacter* sp*, Enterobacter* sp, *S. aureus* and *Klebsiella* sp*.* (Jentsch and Swanevelder, 2019). The researchers found that even though commensal and pathogenic bacteria were detected, there were no reports of death or sepsis (Jentsch and Swanevelder, 2019).

2.9 Infection prevention and control

The need for MRSA prevention is crucial in health-care facilities. In England, infection prevention and control (IPC) for the spread of MRSA resulted in a decrease of infections by implementing improved clinical practice, suitable hand-hygiene, environmental cleaning and improved interventions for high risk infection procedures (Duerden *et al.*, 2015). Not only were disinfection protocols necessary, but so was screening of patients, which became routine procedure in many English hospitals (Duerden *et al.*, 2015).

In the USA a program was employed to control the spread of MRSA. The spread of MRSA in the USA was high, and the Virginia Medical Centre moved form a passive detection to active surveillance (Mckenna, 2010). This campaign employed two individuals as hand-hygiene observers to monitor hand hygiene on a daily basis in the facility (McKenna, 2010). This ensured that all professional staff at the hospital engaged in proper hand hygiene at all times. Individuals who did not comply with proper hand hygiene were notified of their transgression, signed by the medical officer at that time (McKenna, 2010). Over time, the rate of MRSA infection dropped drastically; with 193 cases of MRSA in 2006, 170 cases in 2007 and by 2008, only 123 cases were seen. The company saved around one million dollars in three years (McKenna, 2010).

A 10-year retrospective study conducted in United States Veterans Affairs Hospitals, looked at the association between safety measures and MRSA transmission. This study was conducted by introducing parameters where the value of contact precautions were estimated and the difference in MRSA transmissibility with patients with contact precautions compared to those patients without precautions (Khader *et al.*, 2021). The researchers found that with the introduction of contact safety measures, the transmission of MRSA between individuals was reduced (Khader *et al.*, 2021). This outcome was achieved by employing a veteran affairs MRSA prevention initiative, where guidelines were put in place to determine whether patients required contact safety measures or not. This initiative resulted in a reduction (two-fold) in MRSA transmission (Khader *et al.*, 2021).

The question arises as to the methods used for the decolonization of MRSA positive patients or carriers to prevent the onward spread of MRSA. One such study looked at the use of the common decolonizing agents chlorhexidine gluconate (CHG) and mupirocin in an intensive care unit (ICU) ward. These decolonizing agents were used as CHG baths as well as nasal mupirocin. Using a mathematical model of an ICU ward, this study used a stochastic method to estimate the parameters for assumed MRSA patients decolonized with CHG and mupirocin. It was thus estimated that even though chlorhexidine was effective against MRSA, with a 15% effectiveness, the ideal mathematical model estimated a 30% effectiveness against MRSA (Lofgren *et al.*, no date). The study concluded that even though both chlorhexidine and mupirocin are effective against MRSA, there is room for improvement regarding its effectiveness. In addition, researchers in Southern California conducted a study where two groups of patients were selected; post discharge MRSA decolonization with hygiene education, and education only. The results showed there was a 30% lower risk of MRSA with both decolonization and education, compared to just education alone (Huang *et al.*, 2019).

2.10 The effect of MRSA on the human population

In general, infectious diseases affects the human population globally, but in a study conducted by Fonkwo, he states the following needs to be taken into consideration when looking into the future: “the relationship between increasing microbial resistance and scientific efforts to develop new antibiotics and vaccines; the future of developing and transitional economies, especially with regard to improving the basic quality of life for the poorest people; and the success of global and national efforts to create effective systems of surveillance and response” (Fonkwo, 2008). In 2000, Galloway identified that MRSA was found in large cities and had the ability to spread due to industrialization and urbanization (Galloway, 2000). Her study indicated that MRSA not only affected the poorer settlements, but the affluent and she thus suggested that proper education, MRSA screening and vigilance be implemented (Galloway, 2000). Antonanzas *et al. (2015)* found the cost of MRSA in hospital patients were one and a half to three times greater than simple *S. aureus* infections due to the length of stay in hospital by MRSA positive patients (Antonanzas et al., 2015).

2.11 Coronavirus disease 2019 and MRSA

With the rise of the Coronavirus disease 2019 (COVID-19) pandemic, the call for the use of antimicrobials have increased worldwide (Dewey *et al.*, 2021). Mahalmani *et al.* (2021) indicated the use of antimicrobials may aggravate the issue of antimicrobial resistance and could create an increase in the emergence of multi-drug resistant bacteria (Mahalmani *et al.*, 2021). Antimicrobial resistance usually occurs naturally, when a microorganism is exposed to a hostile environment, such as that of antibiotics (Ansari et al., 2021). However, the lack of research and information about antibiotic and disinfectant use during the pandemic and the impact on the emergence of antimicrobial resistance is still unknown (Miranda *et al.*, 2020).

A recent study conducted in Iran attempted to show the correlation between COVID-19 and bacterial coinfections. The study showed that at least 12% of all COVID-19 positive patients admitted were positive for a bacterial coinfection, with 14% of those patients being positive for MRSA (Mahmoudi, 2020). Mahmoudi (2020) showed that even though the bacterial coinfection with COVID-19 was infrequent, the increase in hospital admissions with these multi-drug resistant bacteria would result in bacterial transmission to healthcare workers and thus an increase in the use of antibiotics (Mahmoudi, 2020). Not only did this study advise against the misuse of antibiotics, it suggested that increased use of disinfectants by hospital staff and the use of biocidal agents in non-healthcare settings could lead to the increase in antimicrobial resistance (Mahmoudi, 2020).

However, a study conducted by Baker *et al. (2021)*, showed that healthcare associated infections were on the rise with the advent of COVID-19. The study conducted in the USA showed a 60% increase in blood stream infections and a 43% increase in catheter-associated infections (Baker *et al.*, 2021). The investigators found a 44% increase in MRSA infections. A similar study conducted by O'Toole (2021) found that the detection of MRSA rose from 0.6% to 5.7% in respiratory cultures of COVID-19 positive patients (O'Toole, 2021). The researcher reasoned that MRSA was more likely to have been hospital-acquired in COVID-19 positive patients than community-acquired (O'Toole, 2021). The surge in COVID-19 infections has adversely affected infection rates in hospital, whereas prior to COVID-19, hospital infection rates were on the decline due to the focus on surveillance and infection control (Baker *et al.*, 2021). Baker *et al. (2021)* suggested the reason for the increase in bacterial infections were due to the impact of the pandemic where there was added burden for COVID-19 patient care, disruption of routine practice as well as lapses in infection prevention and control (Baker *et al.*, 2021). Baker *et al* (2021) stressed the importance of implementing strategies for infection and prevention control during pandemics such as COVID-19 (Baker *et al.*, 2021). With the future looking bleak regarding antimicrobial resistance, Miranda *et al* (2020) emphasized that it is a worldwide problem. It plays a role in social, economic and healthcare arenas and with the impact of the COVID-19 pandemic; this situation could only worsen (Miranda *et al.*, 2020).

2.12 Conclusion

Methicillin-resistant *Staphylococcus aureus* has left its mark worldwide. From hospitals, to communities outside of healthcare settings, humans have been affected. Humans have been infected with MRSA from simply being exposed to another person who is an MRSA carrier, by touching a TV remote in an old age home or a bed rail in a hospital. In these hospitals, blood products for blood transfusions are somewhat common, but rarely thought about as a carrier of MRSA. There is a real risk that the movement of blood using SANBS hamper system could be contributing to the spread of MRSA and other bacteria between patients, hospital wards, and SANBS facilities in South Africa.

In this Chapter, we looked at the characteristics of MRSA and the effect this bacterium has in healthcare and non-healthcare setting. The need for testing in blood services and the significant contribution of new knowledge on the role of external healthcare delivery partners in the spread of MRSA and other pathogens have been described.

Review of methods used to isolate MRSA

Principle of CHROMagar MRSA: CHROMagar MRSA allows for accurate detection of MRSA with an increased level of sensitivity than oxacillin containing media. The presence of a rose to mauve color colony gives a positive MRSA status. CHROMagar™ MRSA allows for the direct detection and identification of MRSA through the combination of chromogenic substrates and cefoxitin. MRSA strains will grow in the presence of cefoxitin and produce mauve colonies resulting from hydrolysis of the chromogenic substrate. Additional selective agents are added for the suppression of gram-negative organisms, yeast and some other gram positive cocci. Bacteria other than MRSA may use other chromogenic substrates in the medium resulting in the growth of colonies that are not mauve.

The following Chapter 3 provides a detailed description of the study design and methods applied for data collection.

CHAPTER THREE: METHODS

Permission was obtained from South African National Blood Service (SANBS) to perform this study where the protocol was approved by SANBS Scientific Review Committee. Ethics approval was obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) (Ethics Clearance Certificate No: BREC/00000743/2019) and SANBS Human Research Ethics Committee (HREC) (Clearance Certificate No: 2019/0493). The flow diagram (Fig. 3.1) below shows the process followed for the completion of the study.

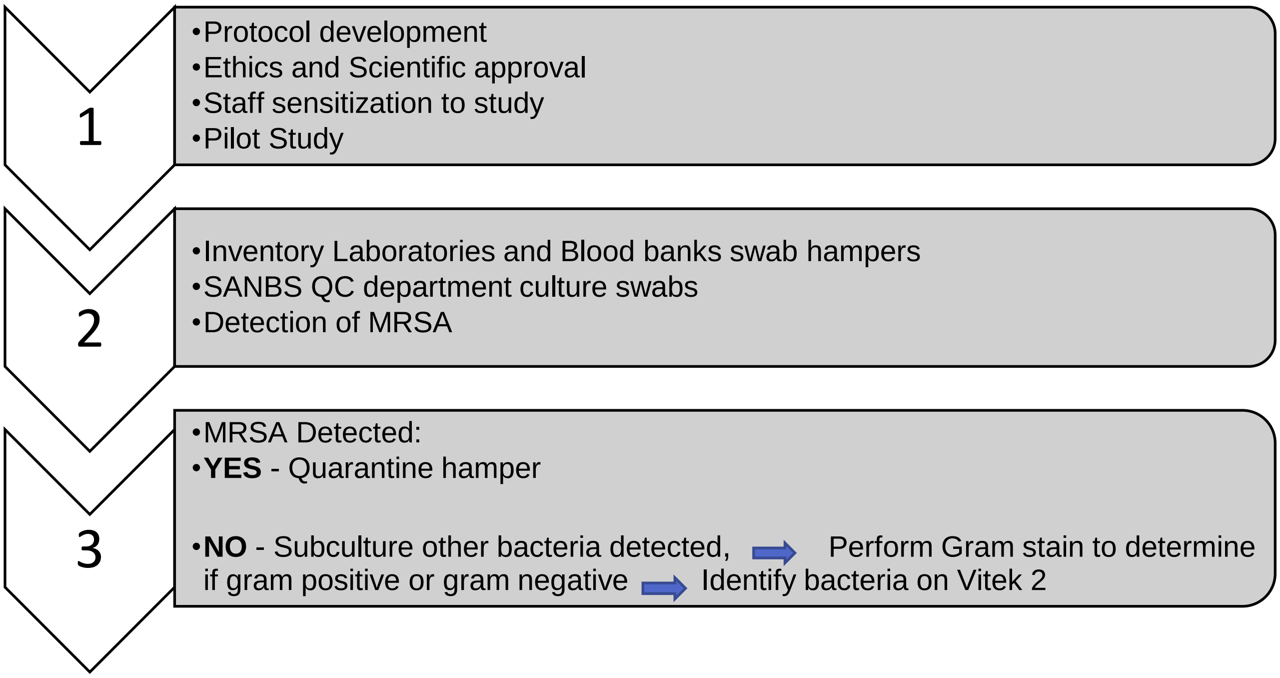

Figure 2.1: Flow Diagram of Study Plan

3.1 Study setting

South African National Blood Service is the larger of two blood transfusion services in South Africa, supplying blood to eight of the nine provinces (SANBS, 11 February 2022, https://sanbs.org.za/about-us/). The Western Cape Blood Service supplies blood to the remaining province. Within SANBS there are seven operational zones: Eastern Cape Zone which covers Eastern Cape Province, Egoli Zone which covers the Gauteng Province, Kwa-Zulu Natal (KZN) Zone, which covers the Kwa-Zulu Natal Province, the Mpumalanga Zone which covers Mpumalanga Province, Northern Zone which covers Limpopo Province, Pretoria and Rustenburg, the Free State/Northern Zone which covers Free State and Northern Cape Province and lastly the Vaal Zone which covers Vanderbyl Park, Klerksdorp and Mafikeng.

A map of South Africa (Fig. 3.2) below shows the SANBS blood banks in their respective zones.

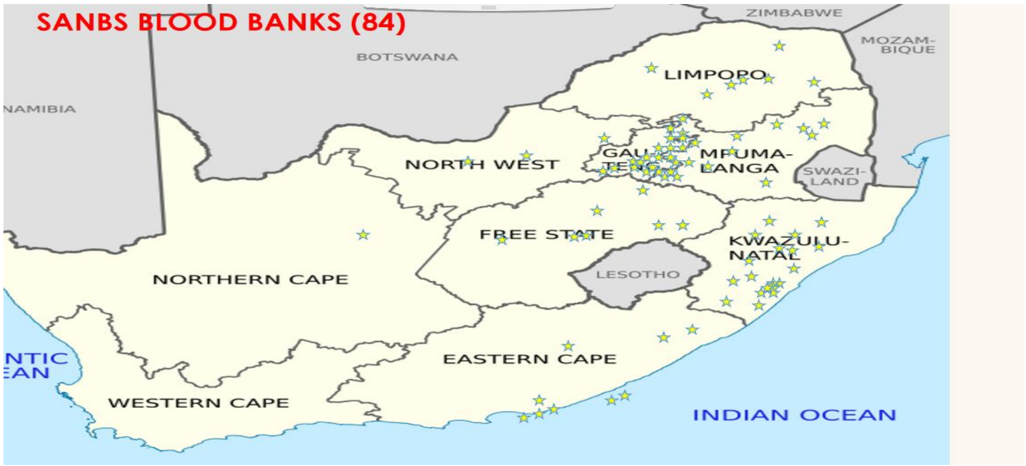

Figure 3.2: Map of South Africa with the SANBS blood banks plotted

These zones are responsible for collecting blood at the various donor clinics, as well as the production and distribution of blood and blood products in their respective areas within South Africa. Donated blood is collected at the various SANBS donor clinics in the South Africa and thereafter placed in a validated hamper for transport to SANBS Processing Laboratories (WPBTS and SANBS, 2017). The Processing laboratories are situated in each of SANBS zones. Once the donated units have been processed, tested and labelled, they are moved to the inventory laboratories. These laboratories are situated in the same vicinity as the Processing laboratories. The blood is stored in the inventory laboratory in temperature-controlled refrigerators until requested for by Blood banks or by a routine order. The blood is transported to the blood banks in a validated blood product hamper (WPBTS and SANBS, 2017). Once the blood is received at the blood bank, the hamper is emptied and returned to the inventory lab. The blood type is re-grouped, and the units are placed in temperature-controlled refrigerators (WPBTS and SANBS, 2017). When a doctor requests for a unit of blood, a crossmatch is received, and the unit tested against the patient blood sample. The crossmatched units remain in the blood bank refrigerator until pick up by the courier company or doctor himself.

3.2 Methodology

3.2.1 Study Design

A cross-sectional study was performed to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) on blood product hampers at SANBS between January 2020 to June 2020. Due to the Coronavirus disease 2019 (COVID-19) pandemic, the study was delayed and conducted between August 2020 to May 2021.

3.2.2 Study Population

The target population were all blood product hampers used for the transport of blood from inventory laboratories to blood bank laboratories as well as from blood bank laboratories to inventory laboratories. The accessible population were blood product hampers which were sampled at specified dates from iInventory laboratories going to blood bank laboratories as well as from blood bank laboratories going to inventory laboratories. Hampers used for the transport of donated blood from donor clinics to the processing laboratories were excluded.

3.2.3 Sample Selection

i.Inclusion Criteria

All blood product hampers used for the transport of blood from inventory laboratories to blood bank laboratories as well as from blood bank laboratories to inventory laboratories.

ii.Exclusion Criteria

All blood product hampers which were not used for blood and blood products, tubes received without swabs immersed in the preservative liquid, swabs received with incorrect dates or nonsensical dates and swabs received with incomplete information such as date swabbed, origin of swab.

3.2.4 Pilot Study

In order to assess study logistics and identify any weakness, a pilot study was conducted by the researcher where 10 hampers were tested. Five hampers were tested at the inventory laboratory, and five hampers tested at the blood banks. The pilot study will be mentioned in the write up of this study, but the results were not analysed.

3.3 Study setup and preparation

A meeting was held virtually with the managers and supervisors of the blood banks and inventory laboratories, where the study was to be conducted. The study was explained in detail by the researcher. The researcher created and recorded a video which showed the process of sample collection and transport of the hamper swabbing. This was URL (Uniform Resource Locators) linked and made available to SANBS Blood Bank Managers, SANBS Blood Bank Supervisors, SANBS inventory Heads of Departments as well as SANBS Zone Managers. For those individuals who could not view the video, a detailed procedure was developed by the researcher and provided. A schedule was developed for each zones' inventory laboratory and blood bank where blood product hampers were swabbed. This included date and time allocations for the swabbing of the hampers.

Unfortunately, at this time, the Coronavirus disease 2019 (COVID-19) pandemic spread to South Africa and caused the study to be delayed. The staff complement at the sites who were meant to swab and test the swabs were reduced, and the risk of COVID-19 transmission by the study procedures were deemed to be too high for the study to continue. Of importance to this study is the implementation of stringent infection prevention and control measures across all areas of SANBS. These included frequent use of hand sanitizers, wiping down of all surfaces with 70% alcohol solutions, the wearing of face masks, segregation of shifts in laboratories and daily COVID-19 symptom screening. The study commenced once the COVID-19 pandemic eased in the country.

3.4 Measurement

3.4.1 Sample collection procedures

A Sigma-Transwab containing liquid Amie's transport medium was used for the swabbing of the blood product hamper (Fig. 3.3).

Figure 3.3 Sigma-Transwab containing Liquid Amie's for MRSA detection

This swab is suitable for aerobic, anaerobic and fastidious microorganisms, and can be transported at ambient or refrigerator temperatures within 48 hours. The liquid medium is based on the original formulation of Amie's, but without charcoal. It can be used immediately for Gram stains at the time of collecting the specimen. Using one Sigma-Transwab, the blue blood product hampers were swabbed on the outer blue handles (Fig. 3.4), inside the hamper polystyrene (Fig. 3.5) and the base of the hamper (Fig. 3.6).

Figure 3.4: Blue blood product hamper showing the outer blue handles that were swabbed

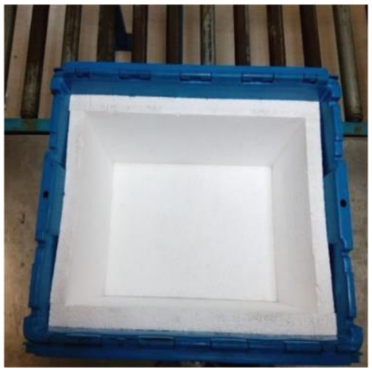

Figure 3.5: Blue blood product hamper showing the inside of the hamper that were swabbed

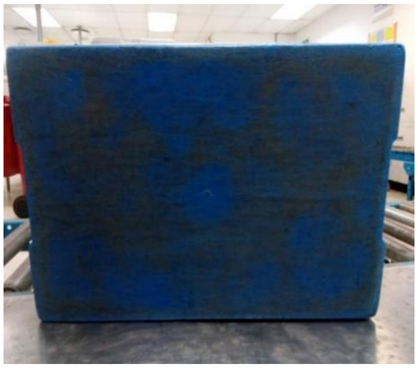

Figure 3.6: Blue blood product hamper showing the base of the hamper that were swabbed

The areas swabbed on the hamper are frequent touch areas, as staff are required to open hampers to either remove or pack blood and blood products. The base of the hamper was swabbed as these hampers are moved to and from various areas and may be placed on the floor, workbench or trolleys.

The swab was placed back into the tube with the cotton bud immersed in the liquid medium for preservation of bacteria. A unique number (barcode) was allocated to the hamper and the swab that was used for swabbing. The last three numbers of the barcode were transcribed onto the hamper with a permanent marker for traceability purposes. The unique number was scanned into an Excel worksheet for traceability purposes and was used on the agar plates for culture as well as the raw data. If at any time the hamper was previously barcoded, the hamper was not numbered again, instead the same number was used, the barcode on the swab and documentation were handwritten. The date, origin and destination of the blood product hampers were documented.

Consecutive sampling was conducted with the first 10 hampers leaving the inventory laboratory being swabbed. These hampers were swabbed by trained SANBS staff just before the hampers were packed with blood and blood products. At the blood banks, all hampers received were swabbed until such time that the sample number was achieved. These hampers were swabbed by trained SANBS staff just before pick-up for return to the inventory lab. All swabs from the inventory and blood banks were placed in a plastic bag together with the documentation, remaining barcodes and placed in a hamper at room temperature (20°C - 24°C). This hamper was labelled and shipped to SANBS Quality Control (QC) department in Constantia Kloof using overnight transport.

3.4.2 *Tests conducted for the isolation of MRSA*

I. Plating of chromogenic agar

Upon receipt at SANBS QC department in Constantia Kloof swabs were inoculated onto the CHROMagar MRSA (Paris, France). The CHROMagar MRSA plates were allowed to reach room temperature (20°C - 24°C) before inoculation. *S. aureus* American Type Culture Collection (ATCC) cultures were used: ATCCBAA1026 (MRSA) and *Staphylococcus aureus* ATCCBAA977 were used for positive and negative QC respectively and inoculated onto CHROMagar MRSA plates with every batch of tests ran. Once the package was received, which included the labelled swab, raw data worksheet and barcode/s, the barcode was checked to confirm if it corresponded to the swab, barcode and raw data worksheet. Using the barcode received in the package, the agar plate was labelled accordingly. The swab was removed from the packaging and using the streak method was inoculated onto the agar plate. Test and QC plates were incubated (Memmert Incubator, Merck, Germany) inverted at 37°C under aerobic conditions.

Plates were checked for growth after incubation of 24 and 48 hours and documented onto the raw data worksheet as well as electronic Excel worksheet. The presence of rose or mauve coloured colonies was recorded as positive MRSA status. If MRSA was detected on a hamper, that hamper was removed from the site and quarantined. Original documentation was sent to the researcher via SANBS internal mail.

II. Bacterial contaminant identification:

Due to the absence of MRSA isolation, from the bacterial contaminants seen on the CHROMagar plates, 20 random isolates were identified towards the end of the study. The CHROMagar plates were observed for contaminant colonies (pink, blue, purple and white), as these were the most common colonies observed. 5 colonies of each colour variation was randomly picked and isolated.

III. Gram stain

The contaminant colony was isolated and a gram stain performed to determine if gram positive or gram negative. The Gram stain is based on the differences in the make-up of the cell membranes and cell walls of the two groups. Gram positive organisms (*S. aureus*) contain a cross-linked layer of peptidoglycan that preserves the first dye. Crystal violet was added first, followed by the addition of the mordant, iodine. The crystal violet and iodine formed a complex within the peptidoglycan so that when decolourizer was applied to the cells, the complex remained within the cell, making it appear dark purple. Gram negative organisms do not contain the cross-linked layer of peptidoglycan. Instead, the peptidoglycan is distributed loosely between the inner cell and the outer cell membranes. After the application of the crystal violet and iodine, the complexes are not stuck within the peptidoglycan and the application of the acid-alcohol decolourizer dried out the outer cellular membrane. This caused holes in the membrane and thus removed the complex from the cells. The cells therefore appeared colourless. To colour the cells, a secondary stain, safranin, is added, allowing the gram-negative cells to stain pink (Wiley, Sherwood & Woolverton, 2008).

IV. Vitek analysis

The isolate was sub-cultured onto sheep blood agar and incubated using the Memmert Incubator (Merck, Germany) overnight at 37°C. Another gram stain was performed from the blood agar to determine if gram positive or gram negative. The isolated colony from the blood agar was used to prepare a 0.5 McFarland suspension and was then loaded onto a card/cassette for the Vitek 2-compact (Biomeriex, France) at SANBS QC Laboratory. The Vitek 2 Compact system uses a fluorogenic method for organism identification and a turbidimetric method for susceptibility testing using a 64 well card that is barcoded with information. Information includes card type, expiration date, lot number and unique card identification number. Test kits available include gram negative bacillus identification (ID-GN) and gram positive cocci identification (ID-GP). The Vitek 2 ID-GN card identifies 154 species of *Enterobacteriales* and a select group of glucose non-fermenting gram negative organisms within 10 hours. The Vitek 2 ID-GP card identifies 124 species of staphylococci, streptococci, enterococci and a select group of gram positive organisms within 8 hours or less. The identification levels percentage probability for the Vitek-2 Compact system shows that percentage probability between 96-99 is excellent, 93-95 is very good, 89-92 is good and 85-88 is acceptable (Pincus, n.d). Results were read and documented on the raw data worksheet. Descriptions of the tests used at SANBS are provided in Appendix 5**.**

3.5 Variables

3.5.1 *Predictor variables*

The site of origin of hamper. The hamper could either come from the inventory laboratory or the blood bank laboratory. The zone where the testing took place where a zone could present with cleaner hampers compared to the other zones. Type of laboratory; inventory laboratories versus blood banks. Either laboratory could be found to be cleaner than the other.

3.5.2 Outcome variables

The presence of MRSA. The presence of contaminant bacteria. Contaminant bacteria colony count and type of bacteria detected.

3.5.3 Confounding variables

Zone – A zone could have a different procedure for cleaning of the hampers which could affect the outcome of the study where we find that one zone has a lower prevalence than the rest. Type of Blood Bank Laboratory which could be 8 or 24 hour blood banks. This would indicate whether these are small or big blood banks which would relate to how busy the blood bank is, and the movement of hampers between the blood bank and the inventory lab.

3.6 Sample Size

The sample size was calculated per group of laboratories, i.e. inventory laboratories and then each geographical zone giving eight groups for comparison not only between the inventory laboratories and the blood banks, but between geographical areas. The following statistical parameters were used to arrive at a minimum sample size with a statistical power of 80%. Type 1 (α) error=0.05, type 2 error = 0.2. According to literature, upon environmental screening a 46% MRSA prevalence was detected in ambulance vehicles in Egypt (El-Mokhtar and Hetta, 2018). The sample size table used was provided in the textbook “Designing Clinical Research” by Hulley *et al*. (2006).

Specifically, “Sample size required per group when using the chi-squared statistic or Z test to compare proportions of dichotomous variables” were used. In this study we did not expect a prevalence above 40% and thus probability was calculated as a probability 1 (Proportion of hampers originating from the inventory laboratories expected to have MRSA – P1) of 26 % (0.26) and a probability 2 (the proportion of hampers from blood banks expected to be positive for MRSA - P2) of 46% (0.46), the effect size of 0.20 was calculated (Hulley *et al*., 2006).

The table does not list 26% as an option, so the higher proportion of 30% with a two-sided alpha of 0.05 and a beta of 0.20 was used. This indicated a required sample size of 103 **per group**, which translates to 824 in total for the 8 groups.

3.7 Analytical Approach

3.7.1 Data analysis

Univariate analysis was used to describe and summarise the data, using proportions for categorical variables and mean and standard deviation for continuous variables. Bivariate analysis was used to determine the significance of association and was tested using the chi-square test for categorical variables and t-test for continuous variables.

3.7.2 Bias

Special care of hampers due to the Hawthorne effect where staff took extra care of the blue blood product hampers since they were being tested for bacterial presence could have occurred during the study. In addition, non-differential misclassification (random loss of swabs) during overnight transport from the inventory laboratories to the QC laboratory in Constantia Kloof. This would affect the sample number and affect the outcome of zone prevalence.

In this chapter, the research methods were described together with the study set up and preparation.

The following chapter, Chapter 4, describes the results obtained. Tables, figures and charts were used to highlight the results obtained.

CHAPTER FOUR: RESULTS

All seven zones at South African National Blood Service (SANBS) participated in the study i.e.: Egoli, Kwa-Zulu Natal (KZN), Eastern Cape, Mpumalanga, Northern Cape, Free State and the Vaal zone. All seven inventory laboratories participated; however, out of the 80 blood banks recruited, only 73 participated. Vereeniging blood bank did not participate as white polystyrene hampers were in use instead of the blue blood product hampers, this blood bank was excluded. The remaining six blood banks did not complete the study procedures as requested by the researcher, and swabs were not collected from these sites.

A pilot study was conducted by the researcher where five hampers from the KZN inventory lab and five hampers from a KZN blood bank, were swabbed as per the study protocol. Swabs were packed by the researcher as per the protocol and transported to SANBS Quality Control (QC) Department in Constantia Kloof for culturing and analysis. The pilot study worked well and amendments to the study were not required. The results of the pilot study was not analysed as per the study protocol.

From the 850 hampers planned to be swabbed, 143 (16.8%) hampers were not swabbed as a result of staff not performing the procedure and swaps from 11 hampers were omitted (1.3%) as they did not comply with protocol requirements. A consort diagram (Figure. 4.1) below shows the sample collection and processing of swabs, together with the swabs not collected together with the reasons for non-participation. Exclusion criteria included tubes received without swabs immersed in the preservative liquid where six tubes were received in this manner, swabs received with incorrect dates or nonsensical dates where one swab was received with an erroneous date and swabs received with incomplete information such as date swabbed, origin of swab where two swabs were received with incomplete information.

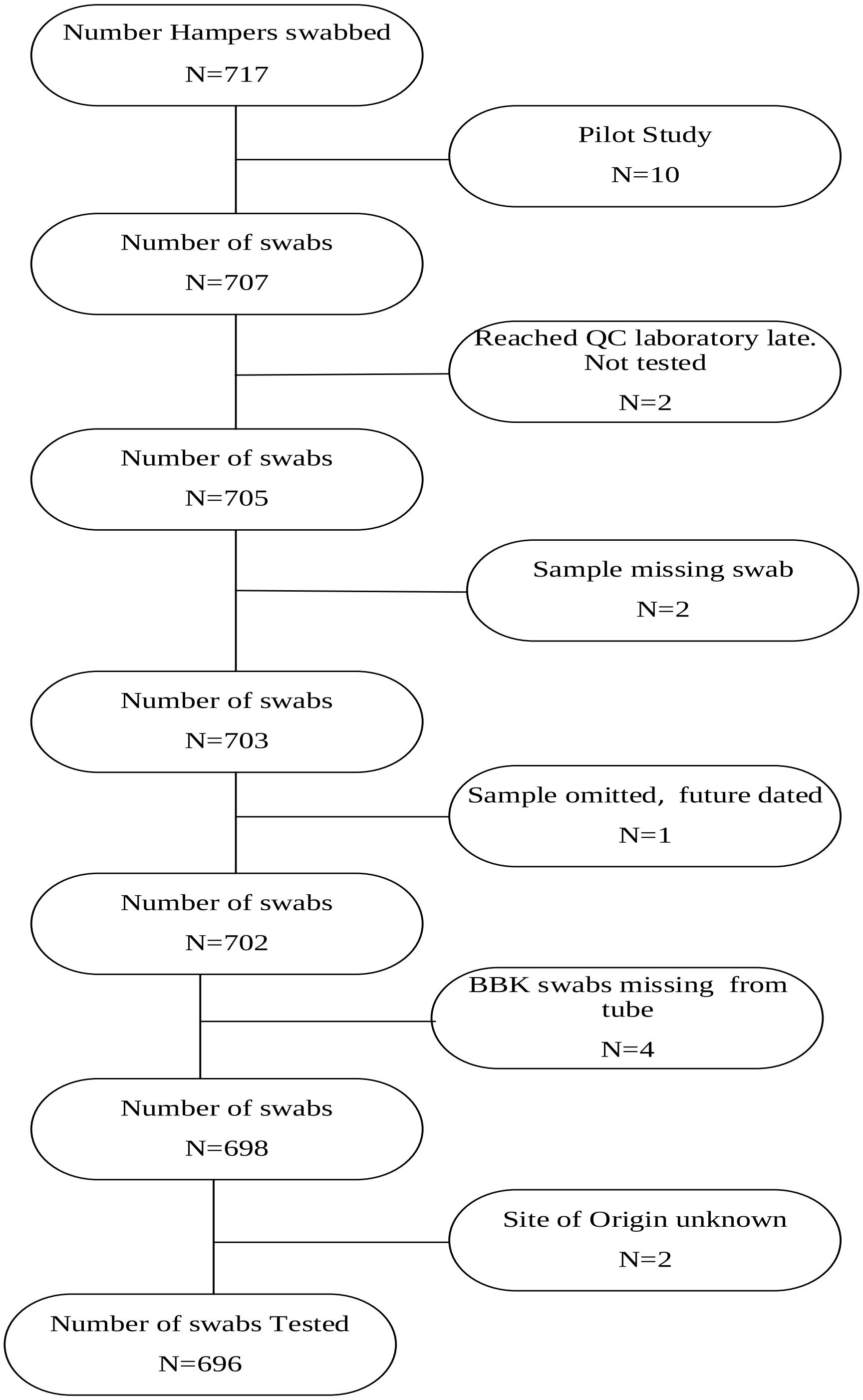

Figure 4.14: Consort diagram for sample collection and processing, indicating the number of swabs not analyzed.

Each zone was given a specified number of hampers that were swabbed, with KZN swabbing the most hampers with a total of 116 (89.9%) and Free State swabbing the least with a total of 87 (73.1%). The inventory laboratories swabbed 104 hampers (99.01%) with the Egoli zone being the only zone not to process 15 swabs. The blood banks swabbed 593 hampers (78.65%) of the hampers, a proportion considerably lower than the inventory laboratories. The inventory laboratories are fewer in number at SANBS, and have a much smaller staff complement compared to the numerous blood banks in the country. They have a more controlled environment and are not as demanding as the blood banks. A total of 696 hampers were processed as per the study protocol (81.9%) and included in the analysis. Results are tabulated in Table 4.1 below.

Table 4.1: Participating zones indicating numbers of hampers swabbed in inventory labs and blood banks.

Zone	Planned number of swabs	Number of swabs meeting inclusion criteria	% of target reached
Eastern Cape	125	101	80.8
Inventory	15	15	100
Blood Bank	110	86	78.2
Egoli	119	92	77.3
Inventory	15	14	93.3
Blood Bank	104	78	75.0
Free State/Northern Cape	119	87	73.1
Inventory	15	15	100
Blood Bank	104	73	70.2
Kwa-Zulu Natal	129	116	89.9
Inventory	15	15	100
Blood Bank	114	101	88.6
Mpumalanga	125	110	88.0
Inventory	15	15	100
Blood Bank	110	95	86.36
Northern	119	96	80.7
Inventory	15	15	100
Blood Bank	104	81	77.88
Vaal	114	94	76.4
Inventory	15	15	100
Blood Bank	108	79	73.1
Overall	850.00	696	81.9

A total of 154 swabs (18.1%) of the hampers were not processed., where 11 swabs were excluded due to not reaching the QC laboratory on time, nonsensical date, site of origin not provided, and swabs not received in preservative solution. The remaining 143 swabs were not processed due to staff not performing the procedure. From the 696 hampers swabbed, methicillin-resistant *Staphylococcus aureus* (MRSA) was not detected (0% detected). Quality control passed with every batch run. See combined results in Table 4.2 below

Table 4.2: MRSA culture results for 696 swabs after 24 hour and 48 hour incubation.

	Incubation Period	
Measurement	24hrs	48hrs
No. of MRSA detected	0	0
Percentage negative QC passed	100%	100%
Percentage positive QC passed	100%	100%

Bacterial growth other than MRSA was observed. A comparison was made between the inventory laboratories and the blood banks at 24 and 48 hours. The proportions are tabulated in Table 4.3.

Table 4.3: Proportion of 696 swabs with bacterial growth at 24hrs and 48hrs

Sites	**Growth at 24 hours N (%)**	**p-value**	**Growth at 48 hours N (%)**	**p-value**
Inventory	57 (52.8%)	0.68	86 (81.1%)	0.23
Blood banks	330 (55.9%)		525 (89%)	

Blood banks showed a greater proportion of bacterial growth compared to the inventory laboratories at both 24 and 48 hours. From the bacterial growth observed the colonies were counted at 24 hours and 48 hours. At 24 hours a total of 22385 colonies were counted. Vaal inventory laboratory was shown to have the highest colony counts with 728 colonies counted and Eastern Cape inventory laboratory with the least colonies counted. Kwa-Zulu Natal blood banks had the highest colony counts with 4233 colonies counted and Eastern Cape blood banks once more having the least number of colonies counted at 1177 colonies. The inventory and blood banks were compared at 24 hours and can be seen in Table 4.4.

Table 4.4: Colony count at 24 hrs. A comparison between inventory laboratories and Blood banks per zone

Number of colonies observed at 24hrs	Inventory N (SD)	Blood Bank N (SD)	p-value
Eastern Cape	1 (NA)	1177 (84.56)	0.2
Egoli	43 (12.95)	2774 (187.23)	0.21
Free State/Northern Cape	702 (53.46)	1944 (280.67)	0.23
Kwa-Zulu Natal	51 (6.80)	4233 (540.70)	0.23
Mpumalanga	233 (31.51)	3071 (27.96)	0.26
Northern	84 (10.81)	3427 (283.31)	0.14
Vaal	728 (82.88)	3917 (226.49)	0.26

At 48 hours a total of 62161 colonies were counted. Egoli inventory laboratory had the least colonies counted with 134 colonies counted and Free State/Northern Cape having the highest number of colonies at 871 colonies counted. KZN blood banks once again presented with the highest number of colonies counted with 10066 colonies counted and Free State/Northern Cape presenting with the least at 5988 colonies counted. The inventory and blood banks were compared at 48 hours and can be seen in Table 4.5:

Table 4.5: Colony count comparison between inventory laboratories and Blood banks per zone at 48 hrs.

Number of colonies observed at 48hrs	Inventory N (SD)	Blood Bank N (SD)	p-value
Eastern Cape	506 (69.57)	7617 (728.79)	0.24
Egoli	134 (25.33)	6863 (507.41)	0.15
Free State/Northern Cape	871 (48.04)	5988 (415.42)	0.15
Kwa-Zulu Natal	306 (33.01)	10066 (995.11)	0.16
Mpumalanga	695 (79.04)	9995 (889.24)	0.14
Northern	188 (17.74)	9897 (1181.28)	0.21
Vaal	735 (82.96)	8300 (585.99)	0.17

At both 24 and 48 hours, the inventory laboratories were presented with a reduced bacterial population compared to the blood banks in the country. Out of all the colonies observed during the study, four common colonies were seen at 24 and 48 hours: pink, purple, blue and white. Due to the absence of MRSA, the study protocol was amended to include random bacterial identification of five isolates of each common contaminant. These isolates were picked randomly, and were subjected to a gram stain to determine if gram positive or gram negative before analysis on the Vitek 2-compact. The gram stain results can be seen in Figure 9:

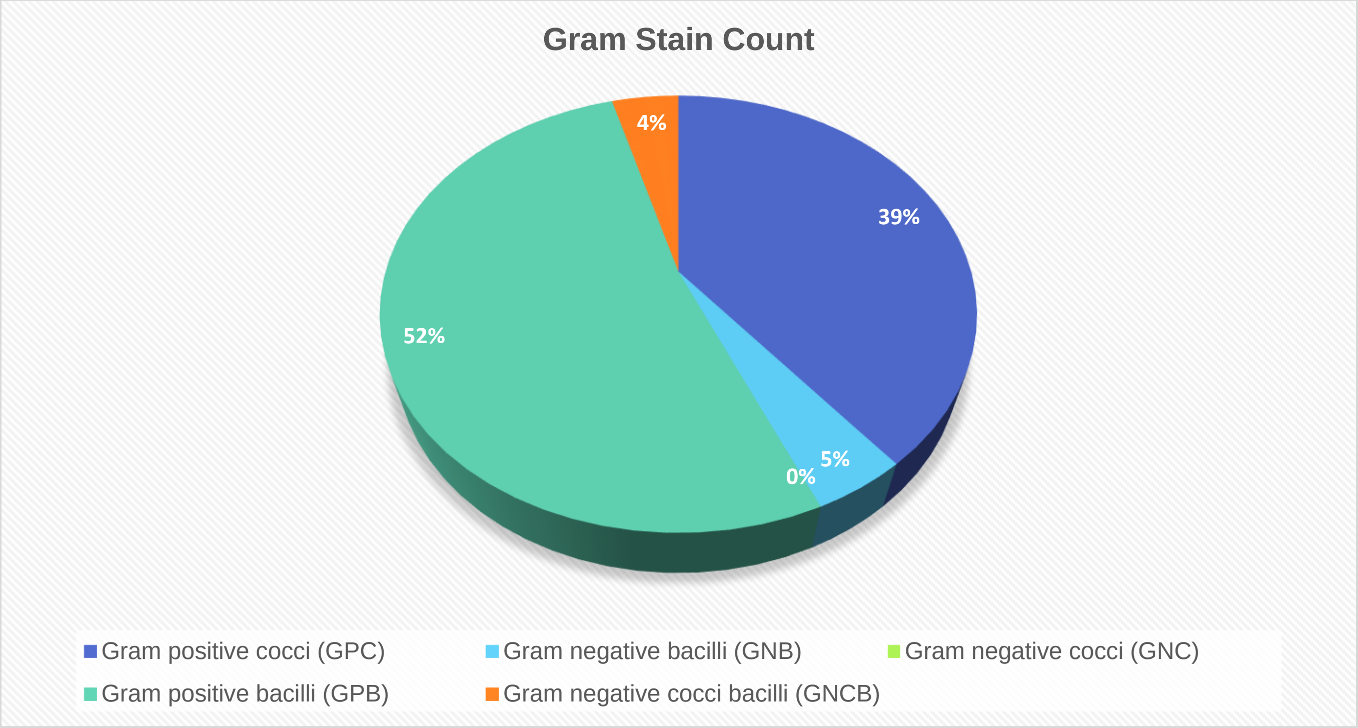

Figure 4.2: Bacterial contamination count using the Gram Stain

The gram stain results showed a majority of gram positive bacilli detection followed closely by gram positive cocci. Gram negative cocci bacilli and gram negative bacilli were detected whilst gram negative cocci were not seen.

The bacterial contaminants were then sub-cultured onto blood agar and allowed to grow overnight. A gram stain was performed and 0.5 McFarland suspension prepared and then loaded onto the Vitek 2-compact for identification. Depending on the gram stain results, the ID-GN and ID-GP kits were used.

Gram positive cocci were observed as grey and white colonies with the most common isolate being *Aerococcus viridans.* The gram positive bacilli were observed as generally purple colonies with the most common isolate being *Rothia dentocariosa,* and gram negative bacilli as blue colonies with *Stenotrophomonas maltophilia* being detected. Results can be seen in Table 4.6 below.

Table 4. 6: Other bacteria isolated from blood product hampers on CHROMagar MRSA.

Organism	Isolates N (%)	Growth characteristics	Gram Stain	%Probability on Vitek 2
S. aureus (not MRSA)	0	No growth	NA	NA
Aerococcus viridans	5 (19.23)	White	Positive cocci	97
Staphylococcus epidermidis	1 (3.85)	White	Positive cocci	97
Kocuria kristinae	3 (11.54)	Grey	Positive cocci	93
Bacillus cereus	1 (3.85)	Purple	Positive bacilli	90
Bacillus megaterium	2 (7.69)	Grey	Positive bacilli	91
Bacillus ceres/thuringiensis/myocoides	4 (15.38)	Purple	Positive bacilli	95
Bacillus clausii	1 (3.85)	Blue	Positive bacilli	85
Bacillus smithii	1 (3.85)	Pink/brown	Positive bacilli	90
Rothia dentocariosa	5 (19.23)	Brown	Positive bacilli	85
Gardnerella vaginalis	1 (3.85)	Brown	Negative cocci bacilli	91
Stenotrophomonas maltophilia	2 (7.69)	Blue	Negative bacilli	99

This chapter reviewed the results obtained using univariate and bivariate analysis. The main outcome looked at the prevalence of MRSA as well as other bacteria on SANBS blue blood product hampers, which carry blood to and from SANBS inventory laboratories and blood banks.

The following chapter, Chapter 5, will discuss the main and secondary findings obtained in this study.

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

5.1 Main findings

The main aim of this study was to determine whether blood services contribute to the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) and other bacterial pathogens through the blood product hamper system. There were three overall objectives including determining the prevalence of MRSA in the blood service's hamper system, determining the prevalence of other bacteria in the blood service's hamper system and determining whether there is a difference in either MRSA or other bacterial contamination between the movement of hampers between South African National Blood Service (SANBS) laboratories.

The study did not detect any instances of MRSA on the blue blood product hampers, which carry blood and blood products to and from the inventory laboratories and the blood banks. Although this was an unexpected finding, this was observed in the study conducted by Makiela *et al*. (2016), which showed a lack of MRSA in the emergency service helicopters. Another study conducted by Stepanović *et al*. (2008), did not find MRSA in the public transport system in Serbia. They found that although MRSA was not detected in the transport vehicles, methicillin-resistant coagulase negative *Staphylococci* (MRCoNS) was (Stepanović *et al.*, 2008). Emergency service helicopters, the public transport system and our study, where hampers were swabbed, all have one thing in common, transport; where items or people are moved from one place to another. It could be that during the transport, the people involved are not present for long periods of time where the MRSA could be 'left behind' for transmission to the next person.

This study was conducted amidst the Coronavirus disease 2019 (COVID-19) pandemic, where strict, additional cleaning protocols and disinfection of staff and work areas were implemented and closely monitored. During the COVID-19 pandemic, SANBS implemented additional infection and control (IPC) measures, which included guidance on proper handwashing, provision of high-quality disinfectants both for hand sanitizing as well as cleaning of the environment (Fig. 5.1).

Figure5.1: Detergent, sporicidal and medicinal alcohol wipes used at SANBS to prevent bacterial contamination.

The detergents used at SANBS include detergent wipes, sporicidal multi-wipes and medi-wipes. The detergent wipes are used first to clean obvious soiling and for general cleaning (SANBS Standard operating Procedure, 2021). The active ingredient nonylphenol ethoxylate is a non-ionic surfactant and is used as a general detergent (Cairn Group MSDS0035, 2017). The sporicidal multi-use wipes are used after the detergent wipe. It is also be used to clean equipment unless otherwise stated by supplier (SANBS Standard operating Procedure, 2021). The active ingredients include didecyldimethylammonium, potassium carbonate, 2-aminoethanol and propan-2-ol (Cairn Group MSDS0031, 2017). Lastly, medi-wipes are high alcohol wipes used to clean spills and disinfect equipment

(SANBS Standard operating Procedure, 2021). The active ingredients include ethyl alcohol and benzalkonium chloride (Cairn Group M0012, 2017).

Provision of personal protective equipment (PPE), extensive training on the donning and doffing of PPE such as lab coats, gloves and masks via emails, posters and training sessions were made available. Similar measures were implemented by all hospitals and courier services. These factors may have contributed to the lack of MRSA detection during this period. This then indicates that SANBS was able to maintain proper IPC with the staff members during the pandemic and that staff followed the necessary precautions when working with the blue blood product hampers.

However, the absence of MRSA is an indication of a lack of human presence. In most of the studies described in this study, the focus areas were hospitals, ambulances, nursing homes; places where ill people were present in large numbers, whereas South African National Blood Service (SANBS) is none of these. Donors are generally healthy individuals and the hospital patients are never in contact with the blood banks or the hampers themselves. Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to spread via contact between humans and objects (Rocha *et al.*, 2020), and the lack of human contact would generally lead to a lack of MRSA.

5.2 Secondary findings

The presence of bacterial contaminants was detected in this study. However, it is important to note that the other bacteria identified may not be a true reflection of what could have been found on the blood product hampers, since the amendment occurred towards the end of the study. Bivariate analysis shows that the growth of bacteria was greater in the blood banks than in the inventory laboratories, with Blood banks showing 89% growth at 48 hours and inventory laboratories 81.1% growth. This was not an unexpected outcome as the inventory laboratories have a restricted access compared to the blood banks, especially those situated within the hospitals. All inventory laboratories are situated within SANBS facilities, which are private property, and have restricted access. Blood banks are situated within the hospital building or outside the hospital building. In either situation, the number of individuals in the area, as well as the cleanliness of the area would vary greatly in comparison to SANBS inventory laboratories.

Bacterial growth within the zones after 48 hours showed the highest numbers in Kwa-Zulu Natal (KZN) with 14.5% followed closely by Mpumalanga with 14.1%. The Free State/Northern zone showed the least bacterial growth at 11.1%. This differential outcome of bacterial growth could be due to the fact that KZN has more blood banks than the other zones, and therefore more human contact. However, the method of cleaning of the hampers should not be disregarded. Currently a standard operating procedure on how to clean the hampers is not available for the blue blood product hampers. Staff are currently using the detergent wipes and medical wipes to wipe down the hampers upon receipt. This being said, it cannot be certain how the hampers are cleaned at the different sites.

Bivariate analysis using the t-test showed a significant difference in the colony count seen at 48 hours between the inventory laboratories and the Blood banks. This analysis reiterated that the Blood banks had a higher number of bacterial contamination when compared to the inventory laboratories. Gram stain results showed the most common bacteria identified were gram positive bacilli with 52% followed by gram positive cocci with 39%.

Most of the bacteria found were environmental bacteria such as *Aerococcus viridans*, an airborne bacterium (Kerbaugh *et al.*, 1968), and *Bacillus cereus*, *Bacillus megatarium*, *Bacillus thuringiensis, Bacillus clausii* and *Bacilus smithii*. The presence of these bacteria are not at all that surprising, as the swabs were taken from the hamper handles as well as at the base of the blood product hampers. These hampers are moved from the laboratories to the transport vehicles and vice versa. At times the empty blood products hampers are stored outside the SANBS building in the loading zones, before being moved inside for cleaning and re-packing.

Drobniewski *et al* (1993) suggested that *Bacillus cereus* infections are seen with surgery, catheters and shunts, albeit these are rare occurrences (Drobniewski *et al*., 1993). *Bacillus megatarium*, is known as a 'non-pathogenic' bacteria (Bocchi *et al.*, 2020) and *Bacillus thuringiensis* known mainly to be found in soil with little reference to human pathogenesis (Hansen., 2000). Similarly, *Aerococcus viridians* is rarely associated with human pathology (Ezechukwu *et al.*, 2019). With regards to the effect of these bacteria on the blood supply, they have little to no relevance, and are not likely to cause disease in patients.

Other bacteria found were *Kocuria kristinae,* and *Rothia dentocariosa*. *Kocuria kristinae* is a bacterium present on the skin and infections associated with this bacteria are rare but can be found in children, and are generally detected on catheters amongst children and some adults who are immunocompromised (Chen *et al.*, 2015). *Rothia dentocariosa* is a bacteria normally found in the mouth (Fridman *et al.*, 2016) and are associated with endocarditis, although this too, is rare (Boudewijns *et al.*, 2003). These bacteria are distinctively people associated and their presence on the blood products hampers is to be expected, even with the current cleaning protocols. The use of masks during production could assist in decreasing the presence of this bacteria.

Of greater concern is the presence of *Stenotrophomonas maltophilia*; aMDRO) (Brooke, 2012). This bacterium, an environmental waterborne MDRO, is associated with pulmonary infections amongst others. Research has shown that *Stenotrophomonas maltophilia* has been associated as a secondary bacterial infection to COVID-19, although it has not been found to be naturally associated with COVID-19 (Pek *et al*., 2021). This bacteria has been isolated together with *Pseudomonas aerginosa, Escherichia coli, Staphylococcus aureus* and MRSA (Brooke, 2012). In hospitals, *Stenotrophomonas maltophilia* has been known to cause infections in immunocompromised bone marrow transplant patients (Kampmeier *et al.*, 2017) and the fact that it has been detected in a blood product hamper is of great concern.

There was found no difference in MRSA found between the laboratories, however, there was a difference detected in the colony count of the bacterial contamination between the Inventory laboratories and the blood banks. The inventory laboratories had a similar prevalence of the bacterial population amongst each other, but a significantly lower bacterial population when compared to the blood banks. The blood banks showed higher bacterial populations possibly as a result of the nature of blood banks: busy, more staff, various shifts and less control compared to the inventory laboratories.

5.3 Unexpected findings

Gardnerella vaginalis, a bacteria responsible for sexually transmitted disease and the cause of bacterial vaginosis (Schwebke *et al*., 2014) was detected. Although *G. vaginalis* is generally associated with sexual contact, Verstraelen *et al.* (2010) suggests that coital transmission is not always necessary for infection (Verstraelen *et al.*, 2010) and in this case, possibly contamination as a result of improper hand hygiene. Sexually transmitted infections are known to not survive well outside the host and although a small percentage was detected, the presence indicates a problem with hygiene in the work area.

Another unexpected bacterium detected in the study was *S. epidermidis*. This bacterium, as per the CHROMagar used for the detection of MRSA, should have been inhibited. Reasons for the presence of this colony on the agar are unexplainable. A possible contamination from staff members whilst preparing the test suspension may have contributed to the presence of this bacteria in the study. However, there have been cases where *S. epidermidis* was found to be resistant to cefoxitin (Hellmark *et al.,* 2009), the active antibiotic found in CHROMagar.

5.4 Limitations of the study

A few limitations to the study were identified:

> The study was initially designed to detect the prevalence of MRSA. However, since MRSA was not detected, the study protocol was amended to determine the presence of other bacterial contaminants seen. Thus, the other bacteria identified may not be a true reflection of what could have been found on the blood product hampers, since this amendment occurred towards the end of the study. This is a

limitation, as maybe the study should been designed to first detect contaminants before specifically looking for MRSA.

The bacterial contaminants identified were randomly chosen from the agar plates and were not traced to the site of origin (either blood banks or inventory labs). Therefore the site of bacteria origination could not be determined.

As a result of staff not carrying put the study procedure, 18.1% of hampers were not swabbed. Emails and follow-us were conducted during the study by the researcher, however, 100% participation was not achieved. Due to the relatively small sample sizes in each zone, the study may have been somewhat underpowered to detect an effect size of 20.0 as indicated. However, the complete absence of MRSA in either group contradicts the underlying evidence of the sample size calculation and suggests that any potential difference may be so small as to be operationally significant. In addition, consideration should be taken that all inventory laboratories and blood banks at SANBS were recruited throughout the country. As a result of the sample size being too small, data was unable to be analysed to determine if the operating hours made a difference to the amount of bacterial contamination found on the hampers.

The study may have been biased by the COVID-19 pandemic, where strict, additional cleaning measures were implemented leading to a cleaner environment.

Swabs were to be inoculated onto MRSA Chromogenic *Staphylococcus aureus* elite agar (SAID) plates. MRSA Chromogenic *Staphylococcus aureus* agar is made up of nutrient base which contains different peptones. It also contains a chromogenic substrate of α-glucosidase and a mixture of several antibiotics including cefoxitin, which helps the growth of (MRSA) including hetero-resistant strains. The detection of MRSA strains is done by revealing α-glucosidase activity which results in the production of green colonies. This mixture inhibits most bacteria not belonging to the genus *Staphylococcus*, as well as yeasts. However the researcher was unable to procure this agar.

Nevertheless, with the information gathered in this study, there is a clearer indication on the types of bacteria present on our blood product hampers, even with the increased cleaning measures put in place because of COVID-19.

5.5 Implications of the study findings

Given the current situation, significantly influenced by the COVID-19 epidemic and the stringent IPC measures in place, MRSA is seemingly not a threat to SANBS and the blood supply. Furthermore, the cleaning protocols implemented for the blood product hampers seem to be sufficient to prevent the spread of MRSA; however, the fact that one MDRO and one sexually transmitted bacteria was detected, indicates that the current system may benefit from further IPC interventions. In order for this to become a reality at SANBS, hamper washing stations would have to be added in all zones at inventory laboratories and at blood banks. Furthermore, staff would have to be recruited for this job function. The cost implicated in a task such as this would run into millions of rands but, there is a need to minimize the exposure of bacteria to blood and blood products in order to safeguard patients.

The question is, would this expected cost be worth the potential improvement, taking into consideration the bacteria detected in this study? The cost of implementing hamper washing stations in SANBS is high. This increase in cost for such a task would mean a possible increase in other areas of SANBS: cost of blood and blood products, which would directly affect the patient, salaries, increases and bonuses, which would directly affect SANBS staff and cost of job satisfaction. The 2020 South African Haemovigilance Report reported on the bacterial surveillance of blood products, eye serum, stem cells and environmental samples. For the 2020 – 2021 period, fungal growth was detected in eye serum and one area had bacterial contamination above the specified target. In total 93.33% of bacteria detected was gram positive cocci, which is in accordance with the isolated bacteria detected in this study (Hilton *et al*., 2020). Of greater concern is the pathogens detected in stem cell products which included *Staphylococcus aureus* and *Klebsiella pneumoniae;* however, in both instances the products were not released(Hilton *et al*., 2020). Taking this into consideration, the answer is yes, an effective blood product hamper cleaning system should be implemented to safeguard our blood and blood product supply. The bacteria identified in this study indicate that bacteria are present in every aspect of blood movement.

5.6 Recommendations

A system needs to be designed, validated and implemented for the proper cleaning of blood product hampers to minimize bacteria from contaminating the blood supply. In the interim a proper standard operating procedure should be developed on the method required to wipe down the hampers using the detergent wipes. This will allow for consistency in all parts of the country. Furthermore, regular environmental and bacterial checks should be carried out on the hampers, to determine the bacterial load on the hampers. In addition to this education about IPC and reinforcing all cleaning protocols should be considered.

5.7 Conclusion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant driver of patient mortality and morbidity. Not only are patients affected, but the economy as well, as a result of prolonged hospital stays, MRSA decolonization, treatment and follow-up.

South African National Blood Service (SANBS) is an essential part of the healthcare system in South Africa. The blood and blood products prepared by SANBS go to all hospitals and wards in South Africa. This is a possible vector for the transmission and the spread of MRSA. Our study found that, at least during COVID-19, this is not the case. However, the differences in bacterial load between inventory and blood banks, suggests that the more controlled environment of the inventory labs leads to better IPC, which in turns suggests that the systems at the blood banks can and should be improved.

While MRSA was not detected, other potential pathogens were, suggesting that there should be concern regarding blood and blood product hampers and the spread of bacterial contamination. The study shows that the current cleaning procedures for the blood product hampers are adequate, or it could mean that the COVID-19 cleaning procedures and the implementation of a more effective IPC aided in the absence of MRSA and acted as a preventative measure. Determining the presence or lack thereof of MRSA, has definitely raised awareness of MDRO's at the blood service. Additional systems for improvement should be investigated and subjected to a full cost benefit analysis, to determine best way forward.

The study findings have shown that an effective hamper cleaning system is needed to safeguard the austerity of our blood supply. The findings of this study should be taken into consideration throughout all provinces at SANBS, for the consistent and regular cleaning of hampers which carry blood and blood products.

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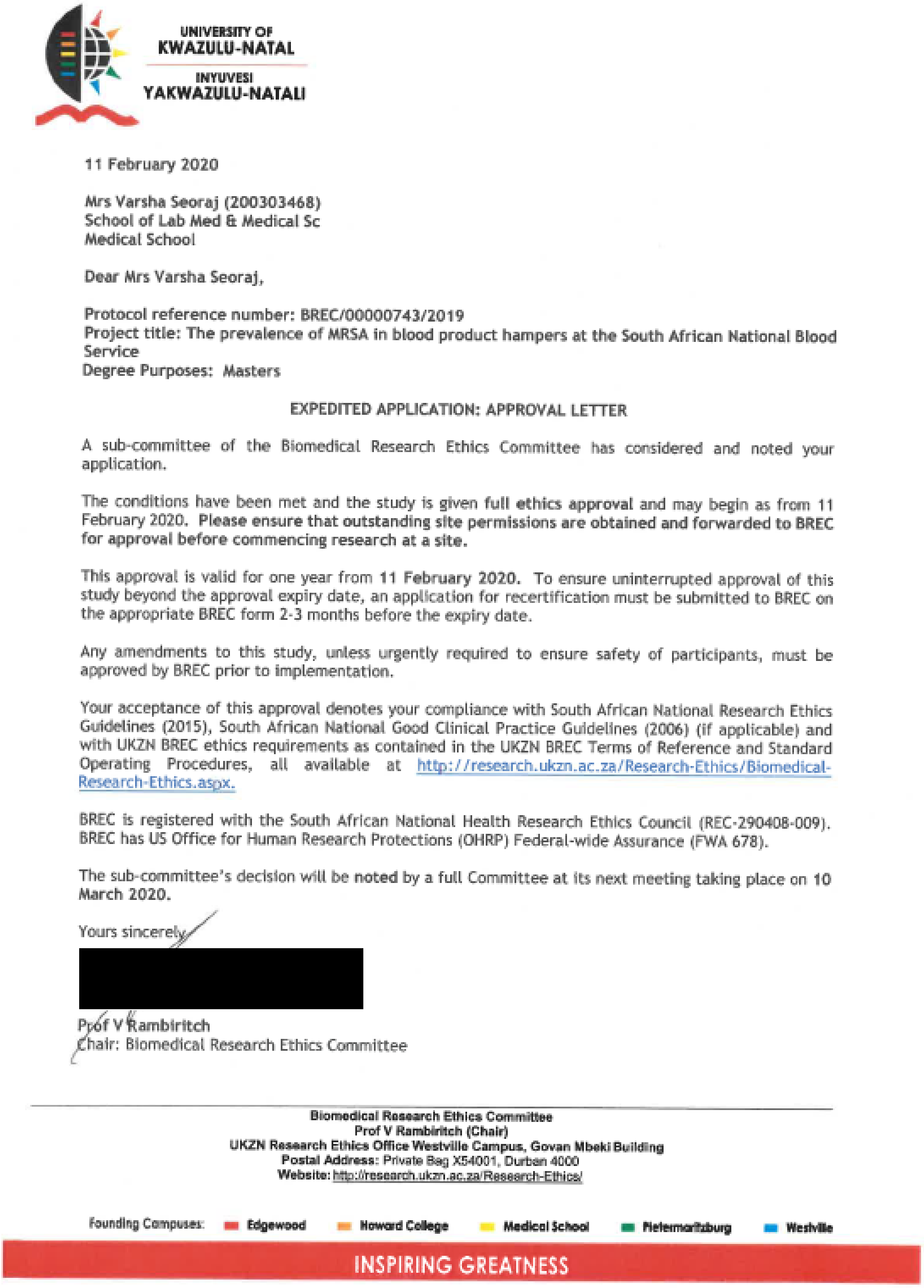
Appendices

Appendix 1 – UKZN Approval Letter



UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

11 February 2020

Mrs Varsha Seoraj (200303468)
School of Lab Med & Medical Sc
Medical School

Dear Mrs Varsha Seoraj,

Protocol reference number: BREC/00000743/2019
Project title: The prevalence of MRSA in blood product hampers at the South African National Blood Service
Degree Purposes: Masters

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given **full ethics approval** and may begin as from 11 February 2020. **Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is valid for one year from **11 February 2020.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on **10 March 2020.**

Yours sincerely

[redacted]

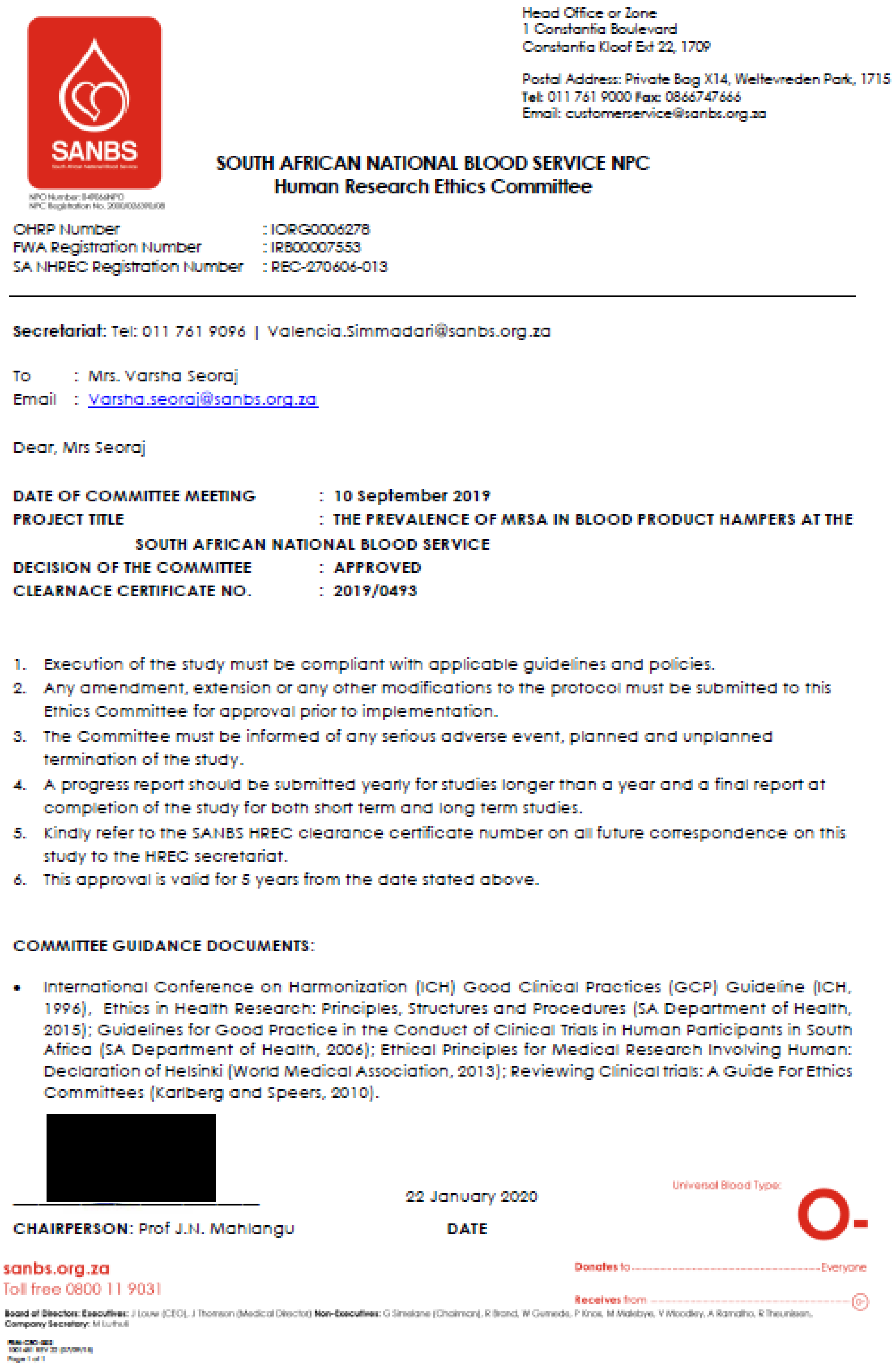
Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Prof V Rambiritch (Chair)
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Website: http://research.ukzn.ac.za/Research-Ethics/

Founding Campuses: 🟥 Edgewood 🟧 Howard College 🟨 Medical School 🟩 Pietermaritzburg 🟦 Westville

INSPIRING GREATNESS

Appendix 2 – SANBS Approval Letter



Head Office or Zone
1 Constantia Boulevard
Constantia Kloof Ext 22, 1709

Postal Address: Private Bag X14, Weltevreden Park, 1715
Tel: 011 761 9000 **Fax:** 0866747666
Email: customerservice@sanbs.org.za

SOUTH AFRICAN NATIONAL BLOOD SERVICE NPC
Human Research Ethics Committee

OHRP Number : IORG0006278
FWA Registration Number : IRB00007553
SA NHREC Registration Number : REC-270606-013

Secretariat: Tel: 011 761 9096 | Valencia.Simmadari@sanbs.org.za

To : Mrs. Varsha Seoraj
Email : Varsha.seoraj@sanbs.org.za

Dear, Mrs Seoraj

DATE OF COMMITTEE MEETING : 10 September 2019
PROJECT TITLE : THE PREVALENCE OF MRSA IN BLOOD PRODUCT HAMPERS AT THE SOUTH AFRICAN NATIONAL BLOOD SERVICE
DECISION OF THE COMMITTEE : APPROVED
CLEARNACE CERTIFICATE NO. : 2019/0493

1. Execution of the study must be compliant with applicable guidelines and policies.
2. Any amendment, extension or any other modifications to the protocol must be submitted to this Ethics Committee for approval prior to implementation.
3. The Committee must be informed of any serious adverse event, planned and unplanned termination of the study.
4. A progress report should be submitted yearly for studies longer than a year and a final report at completion of the study for both short term and long term studies.
5. Kindly refer to the SANBS HREC clearance certificate number on all future correspondence on this study to the HREC secretariat.
6. This approval is valid for 5 years from the date stated above.

COMMITTEE GUIDANCE DOCUMENTS:

- International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guideline (ICH, 1996), Ethics in Health Research: Principles, Structures and Procedures (SA Department of Health, 2015); Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa (SA Department of Health, 2006); Ethical Principles for Medical Research Involving Human: Declaration of Helsinki (World Medical Association, 2013); Reviewing Clinical trials: A Guide For Ethics Committees (Karlberg and Speers, 2010).

[redacted]

CHAIRPERSON: Prof J.N. Mahlangu

22 January 2020
DATE

Universal Blood Type:
O-

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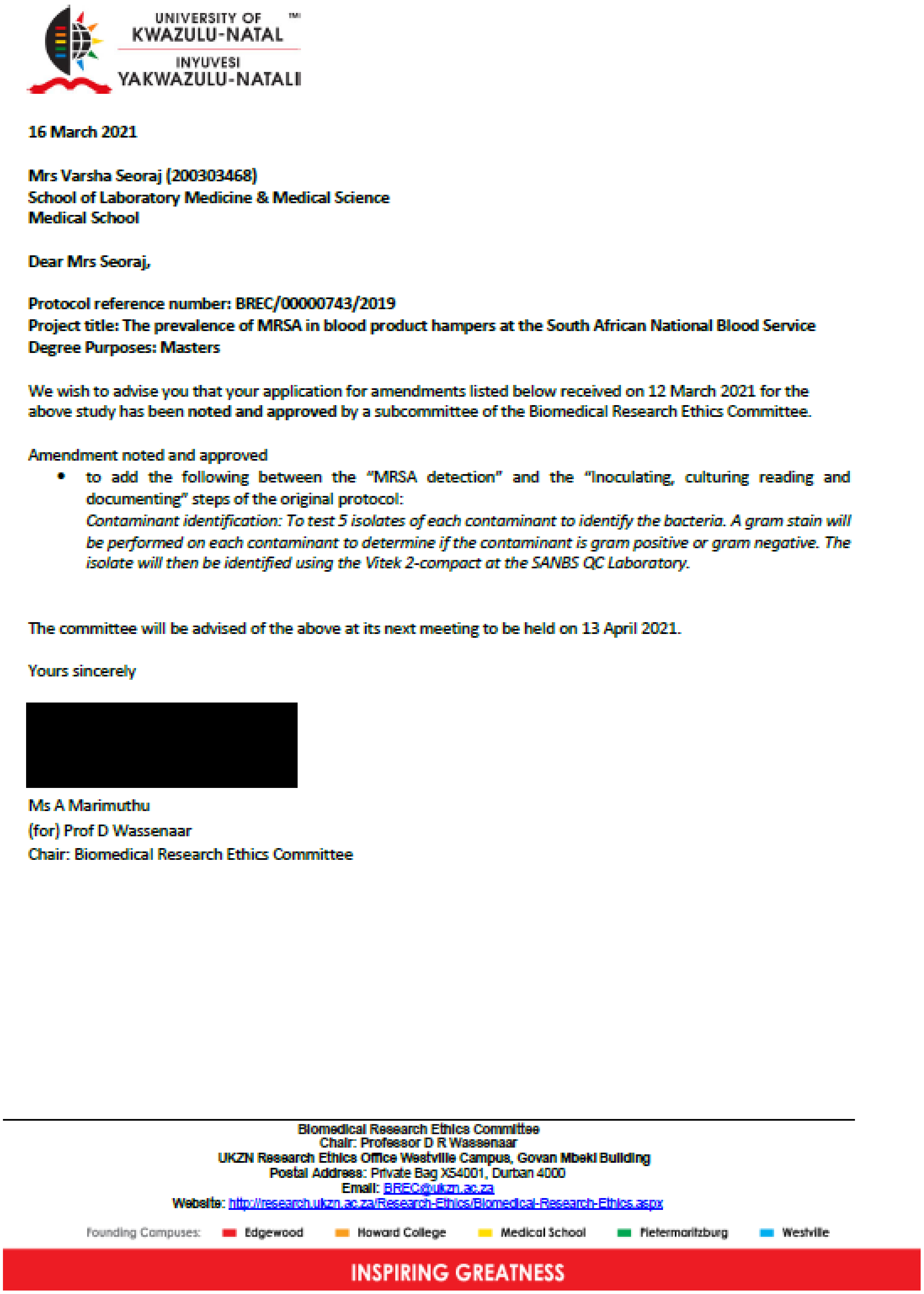
Donates to -- Everyone
Receives from --

Page 1 of 1

Appendix 3 – UKZN Amendment approval letter



UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

16 March 2021

Mrs Varsha Seoraj (200303468)
School of Laboratory Medicine & Medical Science
Medical School

Dear Mrs Seoraj,

Protocol reference number: BREC/00000743/2019
Project title: The prevalence of MRSA in blood product hampers at the South African National Blood Service
Degree Purposes: Masters

We wish to advise you that your application for amendments listed below received on 12 March 2021 for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

Amendment noted and approved

- to add the following between the "MRSA detection" and the "Inoculating, culturing reading and documenting" steps of the original protocol:
  Contaminant identification: To test 5 isolates of each contaminant to identify the bacteria. A gram stain will be performed on each contaminant to determine if the contaminant is gram positive or gram negative. The isolate will then be identified using the Vitek 2-compact at the SANBS QC Laboratory.

The committee will be advised of the above at its next meeting to be held on 13 April 2021.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

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INSPIRING GREATNESS

Appendix 4 – SANBS Amendment approval letter

NPO Number: 049046NPO
NPC Registration No. 2000/026396/08

Head Office or Zone
1 Constantia Boulevard
Constantia Kloof Ext 22, 1709

Postal Address: Private Bag X14, Weltevreden Park, 1715
Tel: 011 761 9000 **Email:** customerservice@sanbs.org.za
www.sanbs.org.za

SOUTH AFRICAN NATIONAL BLOOD SERVICE NPC

Human Research Ethics Committee

OHRP Number : IORG0006278
FWA Registration Number : IRB00007553
SA NHREC Registration Number : REC-270606-013

Secretariat: Tel: 011 761 9096 | Valencia.Simmadari@sanbs.org.za

12th March 2021

To : Mrs. Varsha Seoraj

E-mail : Varsha.Seoraj@sanbs.org.za

Dear Mrs Seoraj,

ACKNOWLEDGEMENT OF RECEIPT OF NOTIFICATION

HREC REFERENCE NUMBER : 2019/0493

PROTOCOL TITLE : **THE PREVALENCE OF MRSA IN BLOOD PRODUCT HAMPERS AT THE SOUTH AFRICAN NATIONAL BLOOD SERVICE**

SANBS HREC hereby acknowledges receipt of your email dated 23rd February 2021, in which HREC was notified of the proposed changes to the above-named protocol.

The amendments to be made to the protocol are as per respective supporting documentation.

Your requests for the above changes were accompanied by the following documents

1. HREC Letter for protocol modification
2. Protocol amendment 19.02.21

These documents were reviewed by the Chairman of SANBS HREC who accepted and approved the proposed changes, subject to SANBS being able to meet the request requirements. All other conditions of the study approval remain the same

Yours sincerely

[redacted]

Valencia Simmadari
SANBS HREC Secretariat

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Receives from .... O-

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Appendix 5: Descriptions of Standard Laboratory Assays

Gram Stain:

Principle:

Bacteria are divided into gram-positive and gram-negative. The Gram stain is based on the differences in the structure of the cell membranes and cell walls of the two groups.

Gram-positive organisms contain a cross-linked layer of peptidoglycan that preserves the first dye, crystal violet, following the addition of the mordant, iodine. The crystal violet and iodine form a complex within the peptidoglycan such that when decolorizer is applied to the cells, the complex remains within the cell, making it appear dark purple.

Gram-negative organisms do not contain the cross-linked layer of peptidoglycan. Here, the peptidoglycan is distributed loosely between the inner cell and the outer cell membranes. After the application of the crystal violet and iodine, the complexes are not trapped within the peptidoglycan and the application of the acid-alcohol decolorizer dehydrates the outer cellular membrane. This causes holes in the membrane and successfully removing the complex from the cells. Thus, the cells appear colorless. To color the cells, a secondary stain, safranin, is added, allowing the gram-negative cells to stain pink.

Method:

Flood the slide with crystal violet and leave for 1 minute.

Wash off the crystal violet and cover slide with iodine solution for 1 minute and
then rinse off with tap water.

Decolourise using Gram's decolouriser until the solvent flows colourless from the slide.

Rinse immediately with tap water to stop decolourisation.

Counterstain using Safranin O solution for 30 seconds. In the event that Safranin is not available Carbol Fuchsin can be used.

Air dry the slide, and examine microscopically under 100 x oil immersion lens.

Stain the control slide using Gram negative organism and Gram positive organism in parallel with each test.

Interpretation of results:

Organisms that are gram stained are identified as follows:

- Gram positive cocci in clusters or in chains – round or oval, purple organisms.
- Gram negative cocci in clusters or in chains – round or oval, pink organisms.
- Gram positive bacilli – large purple rods, some containing spores.
- Gram negative bacilli – pink rods (usually small).
- Yeast – budding oval or round purple organisms

Vitek 2-Compact system

Principle:

The Vitek 2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number. Test kits available include ID-GN (gram negative bacillus identification), ID-GP (gram positive cocci identification), AST-GN (gram negative susceptibility) and AST-GP (gram positive susceptibility). The Vitek 2 ID-GN card identifies 154 species of Enterobacteriaceae and a select group of glucose nonfermenting gram negative organisms within 10 hours. The Vitek 2 ID-GP card identifies 124 species of staphylococci, streptococci, enterococci and a select group of gram positive organisms within 8 hours or less. The Vitek 2 Antimicrobial Susceptibility Tests (AST) are for most clinically significant aerobic gram negative bacilli, Staphylococcus spp., Enterococcus spp., and Streptococcus agalactiae. Susceptibility results are available for bacteria in less than 18 hours.

Method:

Perform daily maintenance and IQC and document

Check DensiCHEK calibration using the four commercial McFarland standards

Place the 0.0 McFarland standards in then press zero to zero the DensiCHEK.

Place the remaining McFarland standards and compare readings against ranges indicated on the package insert then record readings.

Perform identification of isolate from culture plate that is not older than 24 hours (exception made for Anaerobes and slow growing bacteria) by following the steps below:

Make McFarland standard as follows:

- Aseptically dispense 3mls of sterile saline onto Columbia agar plate to check for sterility.
- Aseptically dispense 3mls of sterile saline into the appropriate number of test tubes and zero the DensiCHEK.
- Select well isolated colonies from a primary plate. Use an applicator stick/swab to transfer a sufficient number of colonies to the predisposed saline tube.
- The homogenous organism ID suspension must be equal to the recommended McFarland standard required, see ranges on table 1 below.
- Use DensiCHEK to detect the turbidity required.

Table1: Ranges for McFarland

Organism type	**McFarland standard range**
GNB	0.5-0.63
GPC & Bacillus	0.5-0.63
YEAST	1.8-2.2
AN02/Coryne	2.7-3.3

Table 2: Select ID card as per table below:

ID Card type	**Organism type**
GP card	For Gram Positive organisms
GN card	For Gram Negative organisms (Lactose and non-lactose fermenters)
Yeast card	All yeast like bodies (observed on gram stain)
ANC card	Most anaerobic organisms
Coryne card	All *Corynebacterium species*
BCL card	All organisms of the *Bacillus* family

Place ID card and suspension on the 1st slot of the cassette.

Prepare the Antimicrobial Sensitivity (AST) suspension tube by dilution from the ID tube into another saline tube containing 3ml saline using a pipette.

Aspirate 145µl for Gram negative organisms and 280µl for Gram positive organisms.

Mix well with pipette.

Place the AST card and the suspension tube in the 2nd slot of the cassette.

Program cassette on system:

Load the cassette on the filler drawer of the instrument.

View and interpret results.

Appendix 6

To access the Excel worksheet: Results please click on the link below. This will take you to Google Drive where you will be able to view the results. This document is password protected. Please see password in Rebuttal letter.

https://docs.google.com/spreadsheets/d/190A3xccoGGGi4zrc854YwwK67dWlH-Vv/edit?usp=sharing&ouid=103608352539247159613&rtpof=true&sd=true