

**UNIVERSITY OF GHANA  
COLLEGE OF HEALTH SCIENCES**

**ANTIMICROBIAL RESISTANCE AND GENETIC DIVERSITY OF  
*STAPHYLOCOCCUS AUREUS* FROM SURGICAL SITE INFECTIONS AT TWO  
HOSPITALS IN ACCRA**

**BY  
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## DECLARATION

I hereby declare that this is the product of my own research undertaken under the supervision of Professor Eric Sampane-Donkor and Doctor Beverly Egyir and that references made to other people's work have been duly acknowledged. I also declare that this work has neither been presented in whole nor in part for another degree elsewhere.



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## **DEDICATION**

This work is dedicated to my family and to Mr. Kwesi Tipong-Annor Kumi.

## **ACKNOWLEDGEMENT**

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## ABSTRACT

### Background

Surgical site infections (SSIs) are the most common healthcare-associated infections affecting surgical patients. Such infections are often caused by methicillin-susceptible as well as methicillin-resistant *Staphylococcus aureus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to the entire class of beta-lactam antimicrobials; which are largely used in clinical medicine. Patients infected with MRSA therefore have limited therapeutic options, and this may lead to prolonged periods of hospitalisation and high health care cost. In Ghana, information on SSI as well as the occurrence and prevalence of MRSA and MSSA from such infections are scarce. Data on bacteria species recovered from SSI is key for effective surveillance and selection of appropriate antimicrobial therapy. This study therefore, investigated the proportions of MRSA and MSSA using phenotypic and molecular detection tools among patients diagnosed of surgical site infections in two hospitals.

### Aim

The aim of this study was to determine the antimicrobial resistance patterns and molecular characteristics of *Staphylococcus aureus* detected in patients with surgical site infections at the Korle-Bu Teaching Hospital and 37-Military Hospital in Accra.

### Method

This was a hospital-based cross-sectional study conducted from June to November 2018 at the Korle-Bu Teaching Hospital (KBTH) and 37-Military Hospital in Accra. Surgical patients diagnosed of SSI were recruited using the Centers for Disease Control (CDC) case definition for surgical site infection. Patient demographic data (age, sex, type of operation etc.) and wound swabs or aspirates were collected after receiving an informed consent. *S. aureus* was identified using colonial morphology, coagulase testing and the Matrix Assisted Laser Desorption

Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS). Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method; measured zone sizes were interpreted according the CLSI guidelines. Multiplex PCR was performed to detect *mecA* (methicillin-resistant gene), *spa* (*S. aureus* specific gene) and *pvl* (Panton Valentine Leukocidin toxin gene) in the *S. aureus* isolates. Libraries for illumina sequencing were prepared using the Nextera DNA Flex Library preparation kit. Whole genome sequencing was done with the MiSeq Illumina sequencer at Noguchi Memorial Institute for Medical Research (NMIMR). Genomes were assembled using an in-house pipeline; assembled sequences were then uploaded to the centre for genomic epidemiology website (<http://www.genomicepidemiology.org/>) to determine the *spa* types, sequence types and virulence gene content of the *S. aureus* isolates.

## Results

A total of 110 patients were recruited into the study, 34 (12.5%) were male and 76 (69.1%) were female. Patients between the ages of 25-44 years were highest in the number among the patients enrolled. Overall, 13 *S. aureus* isolates (11.8%; 13/110) were recovered and all were resistant to penicillin and susceptible to gentamicin and vancomycin. Cefoxitin resistance (4/13; 30.77%) was detected only in isolates from 37-Military Hospital. On the other hand, tetracycline (46.15%; 6/13) and norfloxacin (15.38%; 2/13) resistance was recorded at both hospitals. Sensitivity of isolates to linezolid (84.62%; 11/13), clindamycin (76.92%; 10/13), rifampicin (92.31%; 12/13), co-trimoxazole (92.31%; 12/13) and erythromycin (53.85%; 7/13) was very high. The four (30.76; 4/13) isolates resistant to cefoxitin (MRSA) and were also positive for *mecA* by PCR. The predominant *S. aureus* genotype found in the study was ST152-t355. The four MRSA detected belonged to ST152-t355 and ST5-t586 clone. Eight (61.53%; 8/13) isolates were positive for the Panton Valentine Leukocidin toxin. Twelve other virulence genes were detected with haemolysin A and B (*hlgA* and *hlgB*) being the most prevalent.

## **Conclusion**

*S. aureus* isolates recovered were genetically diverse. The detection of ST152 MRSA among surgical patients is particularly of interest; this global clone has also been reported in Central Europe, the Balkan, Switzerland and Denmark as a community acquired MRSA. Continuous surveillance may be required to monitor the spread of these pandemic clones in the hospital setting.



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## LIST OF ABBREVIATIONS

|                 |                                                                          |
|-----------------|--------------------------------------------------------------------------|
| ATTC            | American Type Culture Collection                                         |
| $\beta$ -lactam | Beta-lactam                                                              |
| CA-MRSA         | Community-Associated Methicillin Resistant <i>Staphylococcus aureus</i>  |
| CDC             | Centers for Disease and Control                                          |
| CLSI            | Clinical and Laboratory Standards Institute                              |
| CONs            | Coagulase-Negative Staphylococci                                         |
| DNA             | Deoxyribonucleic Acid                                                    |
| ESBL            | Extended Spectrum beta-lactamase                                         |
| EUCAST          | European Committee on Antimicrobial Susceptibility Testing               |
| HAI             | Hospital Acquired Infections                                             |
| HA-MRSA         | Healthcare-Associated Methicillin Resistant <i>Staphylococcus aureus</i> |
| KBTH            | Korle-Bu Teaching Hospital                                               |
| LA-MRSA         | Livestock-Associated Methicillin Resistant <i>Staphylococcus aureus</i>  |
| MDR             | Multi-drug Resistant                                                     |
| MLST            | Multi-locus Sequence Typing                                              |
| MRSA            | Methicillin Resistant <i>Staphylococcus aureus</i>                       |
| MSSA            | Methicillin Susceptible <i>Staphylococcus aureus</i>                     |
| MSCRAMMS        | Microbial Surface Components Recognizing Adhesive Matrix molecules       |
| NHSN            | National Healthcare Safety Network                                       |



|                    |                                                      |
|--------------------|------------------------------------------------------|
| NMIMR              | Noguchi Memorial Institute for Medical Research      |
| NNIS               | National Nosocomial Infections Surveillance          |
| PBP                | Penicillin binding protein                           |
| PBS                | Phosphate Buffered Saline                            |
| PCR                | Polymerase Chain Reaction                            |
| PFGE               | Pulse-field Gel Electrophoresis                      |
| PVL                | Panton-Valentine Leukocidin                          |
| qPCR               | Quantitative Polymerase Chain Reaction               |
| <i>S. aureus</i>   | <i>Staphylococcus aureus</i>                         |
| SCCMEC             | Staphylococcal Cassette Chromosome mec               |
| <i>spa</i>         | Staphylococcal Protein A                             |
| SSI                | Surgical Site Infections                             |
| ST                 | Sequence Type                                        |
| TAE                | Tris-Acetate EDTA                                    |
| TSST-1             | Toxic Shock Syndrome Toxin -1                        |
| VISA               | Vancomycin Intermediate <i>Staphylococcus aureus</i> |
| VRSA               | Vancomycin Resistant <i>Staphylococcus aureus</i>    |
| WGS                | Whole Genome Sequencing                              |
| $\gamma$ hemolysin | Gamma Hemolysins                                     |

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# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

Healthcare-associated infection (HAI) is the most common challenge patients are faced with when they are hospitalized (Zimlichman et al., 2013). Across the globe, 14-33% of HAIs have been classified as Surgical site infections (SSIs) (Allegranzi et al., 2011; Klevens et al., 2007; Labi et al., 2019; Magill et al., 2012; Smyth et al., 2008). The Centers for Disease Control and Prevention (CDC) defines an SSI as an infection that occurs at the site of incision within 30 or 90 days and up to a year (if there is an implant) of a surgical procedure (CDC, 2019). Worldwide, approximately 2-5% of surgical patients have been estimated to develop a surgical site infection (Smyth et al., 2008). SSIs are worth noting because they pose a threat to most patients who undergo surgery by causing delayed wound healing, longer hospitalization, increased readmission rates, increased healthcare cost as well as increased morbidity and mortality (Awad, 2012; Grimble et al., 2001; Klevens et al., 2007; Merkow et al., 2015; Zimlichman et al., 2013). The economic burden alone associated with SSIs have been estimated to be over 3 billion US dollars in the US (Zimlichman et al., 2013).

SSIs are considered preventable infections because most SSIs are caused by microorganisms that are a part of the body's normal flora. However, organisms that are found in the hospital environment are also implicated (Awad, 2012; Bastola et al., 2017). Bacteria species known to cause SSIs include: *Staphylococcus aureus*, coagulase negative Staphylococci, *Enterococcus spp*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Enterobacter spp*, *Acinetobacter spp* and *Proteus spp*. Among these organisms, *Staphylococcus aureus* seems to be the most predominant (Dhar et al., 2014; Lilani et al., 2005; Mpogoro et al., 2014) causing up to 20% of SSIs (Mangram et al., 1999).

*S. aureus* is a normal flora of the skin, nares, throat and vagina. However, its ecological niche is the anterior nares, and at any given time, 20% of the population is persistently colonized (Reygaert, 2013). It has inherent virulence factors like the Pantone-Valentine Leukocidin (PVL) toxin, Protein A, coagulase gamma toxins (HlgA, HlgB and HlgC) and Staphylococcal enterotoxins (SEA, etc.) which enable it to colonize and invade the host tissues (Hetem et al., 2016; Reygaert, 2013). In the early 1940s, penicillin was used to treat staphylococci infections, within a few years of use, resistance of *S. aureus* to penicillin developed. This led to the introduction of methicillin which initially solved the problem. However, two years after its introduction, resistance to methicillin developed as well (Reygaert, 2013). By 1990, 20-35% of *S. aureus* infections in hospitalized patients were caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA). MRSA has become a clinical concern because of its ability to be resistant to several antimicrobials (Kahsay et al., 2014). MRSA strains are classified as healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA), depending on whether they are associated with hospitals, communities, or livestock (David & Daum, 2010; Gorwitz, 2008; Moellering, 2012). Community-associated MRSA tend to be susceptible to other classes of antimicrobials apart from Beta-lactams (David & Daum, 2010) and carry the PVL toxin believed to be associated with increased disease severity and necrosis (Gorwitz, 2008).

Understanding the spread and evolution of *S. aureus* is essential to the development of effective systems to control its spread (Deurenberg & Stobberingh, 2008). Currently there are various typing methods for detecting genetic variation in *S. aureus*. Thus far, pulsed field gel electrophoresis (PFGE) has been the primary method (Deurenberg & Stobberingh, 2008; Rodriguez et al., 2015; Sabat et al., 2013). In more recent times, there has been a shift towards the use of Staphylococcal Protein A (*spa*), Multi-locus sequence typing (MLST) and staphylococcal cassette chromosome (SCC) mec typing (Deurenberg & Stobberingh, 2008).

However, the type of method to use hinges on the problem to be addressed (O'Hara et al., 2016; Sabat et al., 2013).

## **1.2 Problem Statement**

SSIs are the most common healthcare-associated infections and account for up to 33% of all hospital-acquired infections in Ghana (Labi et al., 2019). SSIs remain an important clinical problem as they cause an estimated number of 8,205 deaths each year, prolong hospital stays for patients by at least 11 days, lead to 20% of unplanned readmissions for surgical patients and increases healthcare cost (Awad, 2012; Klevens et al., 2007; Merkow et al., 2015; Zimlichman et al., 2013). In addition, the healthcare system of countries who have limited resources are further burdened (Klevens et al., 2007; Scott, 2009). In the US alone, an increased hospital stay has been shown to lead to an average of \$2900 to \$3000 increase in cost for every SSI (Awad, 2012). In Africa, SSI rates of 2.5% to 30.9% have been reported (Nejad et al., 2011). Postoperative hospital stay associated with SSI has been found to be 13-20 days in Tanzania and Ethiopia (Eriksen et al., 2003; Taye, 2005). Other studies conducted in Ethiopia have reported a mortality rate of 10.8% for patients with SSI compared to 3.9% for uninfected patients (Taye, 2005) and a delayed hospital discharge in 14.7% patients with SSI (Kotisso & Aseffa, 1998).

Saadatian-Elahi, Teyssou, & Vanhems (2008) 20% of SSIs are caused by *S. aureus*. Studies in Africa have also shown that the predominant bacteria isolated from SSIs is *S. aureus* (Bercion et al., 2007; Eriksen et al., 2003; Fehr et al., 2006; Kotisso & Aseffa, 1998). Amongst the *S. aureus* strains, MRSA is rising in prevalence mainly on the African continent, and is implicated in SSIs (Ahmed et al., 2014; Falagas et al., 2013; Iyamba et al., 2014; Kheder et al., 2012.). This is of major concern as the multi-drug resistant nature of MRSA makes control and management of such infections challenging for physicians. The consequence of this is delayed wound healing as well as increased death rates (Cosgrove et al., 2003).

In Ghana, it has been reported that MRSA prevalence is low (Donkor et al., 2019), however, one of the very few studies conducted across the country found a prevalence as high as of 54.7% in wound samples (Odonkor et al., 2012). MRSA has also been identified in blood samples (Dekker et al., 2016) with two studies showing high prevalence of 86.2% (75/87) and 86.5%(64/75) (Karikari et al., 2017; Laryea et al., 2014). Additionally, nasal carriage of MRSA among 10% of surgical patients has been reported (Egyir et al., 2013) and this is worrying as nasal carriage of *S. aureus* is an established risk factor for SSIs (Levy et al., 2013; Mangram et al., 1999). These reports show that MRSA is on the rise and needs to be addressed immediately.

Although *S. aureus* particularly MRSA has both serious economic and health implications, information on its occurrence and spread, resistance patterns and characterization in surgical site is limited throughout Africa and especially in Ghana. Data available in Ghana is on *S. aureus* and MRSA from other samples such as blood, urine, pus and wound but little is known about surgical sites.

### **1.3 Justification**

The outcome of SSI is too high a price to pay and considering the difficulties that arise in treating infections associated with MRSA and the consequences, it is important that its spread is controlled. To be able to do this, effective control and surveillance programs should be put in place to prevent, monitor and regulate the spread of this organism. These measures however rely on accurate information on the epidemiology of the organism (Weller, 2000). In addition, to make prompt decisions on the appropriate antibiotic to use for effective treatment, knowledge about the resistance patterns is essential (Knobler et al., 2003). Early and appropriate treatment when put in place will also help to control the spread of the organism (Knobler et al., 2003). So far, information on surgical site infections as well as the role of *S. aureus* (MRSA and MSSA) principally, in SSI is scarce. This study will therefore fill an

important knowledge gap and help develop potential interventions to reduce the occurrence of MRSA.

#### **1.4 Aim**

The aim of this study is to determine the antimicrobial resistance patterns and molecular characteristics of *Staphylococcus aureus* recovered from patients diagnosed of surgical site infections at the Korle-Bu Teaching Hospital and 37-Military Hospital in Accra.

##### **1.4.1 Specific Objectives**

The specific objectives of the study are to:

- Determine the proportion of patients diagnosed of SSI positive for *S. aureus* and the antimicrobial resistance patterns of the isolates.
- Determine the proportion of MRSA and MSSA among the *S. aureus* isolates.
- Determine the molecular characteristics of *S. aureus* isolates recovered.

## **CHAPTER TWO**

### **2.0 LITERATURE REVIEW**

#### **2.1 Surgical site infections**

An infection of a surgical site is a frequent complication of surgery and the commonest hospital-acquired infection (Awad, 2012). Although infection control practices comprising sterilization techniques, surgical skills and availability of surgical prophylaxis have improved, SSIs persists and remain a problem (CDC, 2019). The term SSI was introduced to replace surgical wound infections in 1992 (Owens & Stoessel, 2008). Surgical site infections form 14-33% of the collection of HAIs (Alleggranzi et al., 2011; Klevens et al., 2007; Labi et al., 2019; Magill et al., 2012; Smyth et al., 2008). SSIs are defined as infections that occur at the site of an incision within 30 or 90 days or up to a year (if there is an implant) of a surgical procedure (CDC, 2019; Mangram et al., 1999). Early efforts to determine the burden of hospital-acquired infections led the CDC to establish the National Nosocomial Infections Surveillance (NNIS) system which was later replaced by the National Healthcare Safety Network (NHSN) to monitor healthcare-associated infections in US acute hospitals (Magill et al., 2012; Mangram et al., 1999). For the reason that SSI definitions were not universal, the CDCs NNIS system developed a standardized criteria for defining SSI which is now being used worldwide (Mangram et al., 1999). This was essential as reporting accurate and interpretable SSI rates depended on consistent and standardized definitions. By the CDC criteria, SSIs are classified as superficial, deep or organ space in relation to the depth of infection (Mangram et al., 1999). Superficial incisional involves the skin and cutaneous tissue and occurs within 30 days. Deep incisional involves deep soft tissues and occurs within 60 or 90 days depending on the surgical procedure performed. Organ space infections occur beyond the deep soft tissues and usually have a 90-day surveillance period. However, superficial infections account for the majority of SSIs and organ space infections are less commonly



encountered. Besides the time period and depth of infection, SSI classification is based on the presenting signs and symptoms like purulent discharge at the site of incision, pain, tenderness, swelling at the site, redness, heat and fever (CDC, 2019; Mangram et al., 1999). Diagnosis of SSI by an attending physician or identification of organisms from the incision by either microbiological testing for treatment or clinical diagnosis also form the basis of classification (CDC, 2019; Mangram et al., 1999). In more recent times, SSI criterion includes having an NHSN operative procedure. This is described as a procedure contained within the NHSN operative code mapping, occurs in the course of an operation where the skin or mucous membrane is incised at least once, or a reoperation via a previous incision and takes place in an operating room (CDC, 2015, 2019).

SSIs are considered preventable infections. Several measures have been suggested to aid in its control and prevention such as appropriate antimicrobial prophylaxis, reducing microbial contamination of surgical instruments by sterilization, putting SSI surveillance in place, proper skin preparation and decontamination of environmental surfaces (Anderson et al., 2014; Mangram et al., 1999). Accordingly, most hospitals, especially in developed countries, have implemented surveillance systems to detect infections and successfully decrease SSIs (Song et al., 2018). The CDC reported a 17% decrease in SSIs between 2008 and 2014 among 10 different procedures (CDC, 2019). This is probably an indication of how well these systems are working.

## **2.2 Magnitude of surgical site Infections**

The CDC's NNIS system reported that between 1986 and 1996, there were 15,523 SSIs following 593,344 operations in acute-care hospitals in the US (Mangram et al., 1999). Then the NHSN reported a 1.9% SSI rate for operations occurring between 2006 and 2008 (Mu et al., 2011). Additionally, a healthcare-associated infection survey conducted by the CDC also found that SSIs among in-patient surgeries in the US in 2011 was approximately 157,500

(Magill et al., 2014). However, the problem of SSI is universal. SSI incidence rates of 2.2%, 1.6%, 1.4%, 1.6% and 2.0% have been found in Europe, Germany, England, France and Portugal respectively (Fan et al., 2014). In mainland China, it has been reported to be 4.5% (Fan et al., 2014). Globally, approximately 2-5% of surgical patients have been estimated to develop a surgical site infection (Smyth et al., 2008). There is, however, a substantial difference between developed countries and developing countries in terms of this problem. In developed countries, SSI is often more controlled probably due to surveillance systems that have been put in place, whereas in developing countries, scarce resources, as well as inadequate infection control practices may contribute to the problem (Allegranzi et al., 2011; Nejad et al., 2011). Data is limited on SSI in Africa, yet, rates of 2.5% to 30.9% have been reported in some parts (Nejad et al., 2011). In Ghana, SSIs have been shown to account for up to 33% of all hospital acquired infections (Labi et al., 2019) and SSI prevalence as high as 39% and 40% have been reported (Ameyaw, 2014; Apanga et al., 2014).

### **2.3 Economic Burden of Surgical Site Infections**

SSI management imposes a significant burden on patients and the healthcare system of a country (Klebens et al., 2007; Scott, 2009). SSIs are also associated with substantial morbidity. Patients with SSIs have to be hospitalized for longer times or readmitted to the hospital and this increases the healthcare cost for them (Awad, 2012; Grimbale et al., 2001; Klebens et al., 2007; Merkow et al., 2015; Zimlichman et al., 2013). Apart from that, individuals with these infections have a higher risk of dying compared with individuals without infections. The economic burden associated with SSIs has been estimated to be over 3 billion US dollars in the US (Zimlichman et al., 2013), \$1,341-\$10922 per patient in England and an average of \$4,544 per day in Europe (Fan et al., 2014). In the US, an increased hospital stay has been shown to lead to an average of \$2900 to \$3000 increase in cost for every SSI (Awad, 2012). Additionally, SSIs have been associated with an estimated 8,205 deaths each

year, prolonged hospital stays of at least 11 days, 20% of unplanned readmissions and increased healthcare cost for patients (Awad, 2012; Klevens et al., 2007; Merkow et al., 2015; Zimlichman et al., 2013). In Africa, information about the economic burden of SSI is scarce. However, a few studies that have been conducted in some countries like Tanzania and Ethiopia, have reported SSI associated postoperative hospital stay of 13-20 days (Eriksen et al., 2003; Taye, 2005), a mortality rate of 10.8% (Taye, 2005) and a delayed hospital discharge in 14.7% SSI patients (Kotisso & Aseffa, 1998). SSIs and their corresponding economic burden have not been studied in Ghana. This may be due to the fact that it has not been acknowledged as a major problem. Much attention has been given to diseases such as malaria, tuberculosis and HIV. However, the impact of SSIs is great and as such surgical patients who develop an SSI need to be given greater attention.

## **2.4 Microbiology of Surgical Site Infections**

SSIs are most often caused by the endogenous flora of the patient and the organism isolated is dependent on the type of surgery performed (Awad, 2012; Mangram et al., 1999; Owens & Stoessel, 2008). However, exogenous sources such as the hospital environment has also been implicated (Awad, 2012; Bastola et al., 2017). In the hospital setting, these organisms may be acquired by direct contact with hospital staff or other patients and improperly sterilized equipment or materials that are used during the surgical operation (Awad, 2012). There are several patient-related and procedure-related factors also contributing to the occurrence of SSIs. Patient-related factors include age, obesity, underlying diseases such as diabetes mellitus (Anderson, 2011; Owens & Stoessel, 2008), and nasal carriage of *S. aureus* (Levy et al., 2013; Mangram et al., 1999). Procedure-related factors include longer duration of surgery (Marimuthu et al., 2016; Mpogoro et al., 2014). Organisms causing infection have not drastically changed over the last two decades (Akinkunmi et al., 2014; Bhave et al., 2016; Lilani et al., 2005; Mangram et al., 1999; Takesue et al., 2017) probably because endogenous

and exogenous organisms continue to be the same. *Staphylococcus aureus*, coagulase-negative Staphylococci, *Enterococcus spp*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Enterobacter spp*, *Acinetobacter spp* and *Proteus spp* are still frequently isolated from SSIs (Mangram et al., 1999). Among these organisms, *Staphylococcus aureus* seems to be the most predominant (Dhar et al., 2014; Lilani et al., 2005; Mpogoro et al., 2014) causing up to 20% of SSIs (Mangram et al., 1999) and nasal colonization being one of the most important risk factors for the development of an SSI (Levy et al., 2013; Mangram et al., 1999; Wertheim et al., 2005). Reports have also shown an increased number of SSIs associated with resistant bacteria such as MRSA (Mangram et al., 1999; Owens & Stoessel, 2008), worsening the burden associated with it.

## **2.5 Overview of *Staphylococcus aureus***

Staphylococci are a genus of Gram-positive cocci bacteria that belong to the order Bacillales and family Staphylococcaceae (Crossley & Archer, 1997). The first identification of Staphylococci was by Alexander Ogston from surgical abscesses in the knee joint (Crossley et al., 2009; Hetem et al., 2016). Staphylococci are viewed as pairs or grape-like clusters under the microscope from which its name was derived (Crossley et al., 2009; Hetem et al., 2016). Generally, they grow well on blood agar but some strains prefer a medium with high salt concentration such as mannitol salt agar. On blood agar, they appear as 2mm golden coloured or white colonies (Crossley et al., 2009; Hetem et al., 2016) and as yellow colonies with yellow zones or pink colonies on mannitol salt agar (Maza et al., 2004). Staphylococci are facultative anaerobes, non-motile and non-spore forming organisms (Hetem et al., 2016). A characteristic feature of Staphylococci is the production of catalase, an enzyme that converts hydrogen peroxide to oxygen and water and distinguishes them from closely related members of the genus Streptococci which are Gram-positive cocci in chains but catalase-negative (Foster, 1996). Most often, they are found as normal flora of the skin, nasopharynx, vagina and mucous

membrane and as such they are not of concern. Yet, a break in the skin or a compromised immune system provides an opportunity to cause disease (Reygaert, 2013). There are over 50 species that have been described so far (Hetem et al., 2016). These species can be divided into two groups based on their ability to clot blood plasma by the production of an enzyme known as coagulase (Foster, 1996). Coagulase producing species comprise *S. aureus*, *S. intermedius*, *S. delphini*, *S. lurae*, *S. hyicus* and *S. schleiferi subsp. Coagulans* (Sasaki et al., 2010). Species that lack this enzyme are known as Coagulase-Negative Staphylococci (CONs) and some members that have been known to cause human infections include: *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, and *S. lugdunensis*. *S. saprophyticus* has been associated with urinary tract infections in young female adults (Crossley et al., 2009)

*Staphylococcus aureus*, a member of the coagulase-positive group is the most pathogenic in this genus (Foster, 1996). During the 1917-1918 Spanish influenza pandemic, it was proposed that most of the deaths were a result of bacterial pneumoniae caused by upper respiratory tract pathogens: *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *S. aureus* (Humphreys, 2018; Morens et al., 2008). Similarly, during the First World War, septic wound infections that occurred were believed to be due to *S. aureus* (Hetem et al., 2016). Although *S. aureus* strains are generally coagulase-positive, some may not produce coagulase (atypical) (Foster, 1996). *S. aureus* is found as normal flora of the skin and nasopharynx. However, it is mainly found in the anterior nares and as such, most people are asymptotically colonized (Hetem et al., 2016; Reygaert, 2013; Wertheim et al., 2005). It has been established that there are three types of carriers namely: persistent carriers, intermittent or non-carriers (Hetem et al., 2016; Wertheim et al., 2005). Approximately 20% of people are persistent carriers while 30% are intermittent carriers (Hetem et al., 2016; Wertheim et al., 2005). Non-carriers are people who rarely harbour *S. aureus* and approximately 50% of healthy people are in this group (Hetem et al., 2016; Wertheim et al., 2005). The principal mode of transmission of this organism is by

person to person contact and contaminated surfaces or hands as they can survive for long periods on such surfaces (Hetem et al., 2016; Reygaert, 2013; Wertheim et al., 2005). Thus, basic hygiene practices such as proper and regular cleaning and disinfecting of surfaces have been proposed for reducing transmission (Siegel et al., 2006). *S. aureus* causes mild to severe infections. Mild infections include skin and soft tissue infections such as cellulitis, carbuncles and scalded skin syndrome. Severe infections include bloodstream infections, meningitis, endocarditis, osteomyelitis, pneumonia as well as conditions like toxic shock syndrome and food poisoning (Crossley et al., 2009; Ostojić, 2008; Tong et al., 2015). This disease process is thought to be facilitated by two mechanisms: first the production of virulence factors or toxins which it inherently has and secondly, colonization that causes invasion of the tissue and eventual destruction (Reygaert, 2013). In addition to its virulence, *S. aureus* has an exceptional ability to acquire resistance to antibiotics and adapt to its changing environment; factors which explain why it has remained a widespread pathogen of medical importance for many centuries (Moellering, 2012).

## **2.6 Virulence Factors of *S. aureus***

*S. aureus* has several toxin genes and virulence factors such as Panton-Valentine Leukocidin (PVL), exfoliative toxins (*eta* and *etb*), a range of enterotoxin genes, hemolysins, Protein A and other enzymes (Hetem et al., 2016; Reygaert, 2013). These are important factors that account for its extraordinary success as a pathogen, as they facilitate adhesion to the surface of eukaryotic cells, allow the organism to evade the immune system, colonize and subsequently cause disease in its host (Hetem et al., 2016; Reygaert, 2013). Virulence factors are found on chromosomes, bacteriophages, plasmids and transposons (Hetem et al., 2016) and a number of these factors need to be expressed at a time for pathogenesis to occur.

Protein A is expressed in almost all *S. aureus* and contributes to the prevention of opsonisation (David & Daum, 2010). It is part of the various surface proteins known as Microbial Surface

Components Recognizing Adhesive Matrix molecules (MSCRAMMS) and encoded by the *spa* gene (Crossley et al., 2009). The *spa* gene has three regions: FC, polymorphic X and C. The FC region attaches to the IgG-FC domain of the host to prevent opsonisation, thus, hindering phagocytosis by the immune system (Asadollahi et al., 2018).

Panton-Valentine Leukocidin (PVL) is probably the most extensively studied toxin. This is an important toxin known to cause necrotizing pneumoniae which is rapidly fatal (Garnier et al., 2006). It is also associated with severe infection and recognized as an indicator of community associated strains of MRSA (Garnier et al., 2006; Müller-Premru et al., 2005). PVL is encoded by two genes *lukS-PV* and *lukF-PV*. These genes act together by binding to specific membrane receptors, forming pores in the membrane of the leukocytes and subsequently causing their destruction (Chambers & DeLeo, 2009). Hence, PVL is linked with enhanced virulence of *S. aureus*.

Exfoliative toxins cause exfoliation of the skin epidermis which is followed by secondary infections. These toxins are not often found in *S. aureus*. Four forms ETA, ETB, ETC and ETD encoded by the genes *eta*, *etb*, *etc* and *etd* have been identified, of which A and B are of utmost importance in human disease (Crossley et al., 2009). ETA and ETB have been associated with neonatal and child staphylococcal scalded skin syndrome (Crossley et al., 2009) while ETD is associated with wound infections (Dufour et al., 2002).

Staphylococcal superantigens consist of numerous toxins believed to be associated with increased virulence of which the staphylococcal enterotoxins are a part (Holtfreter et al., 2007; Tristan et al., 2007). Initially, five serotypes were described, SEA to SEE. However, new enterotoxins are constantly being discovered and named alphabetically according to their order in which they appear. So far, SEG to SEU has been identified (Blaiotta et al., 2006). Enterotoxin A is probably the most studied as it induces a strong proinflammatory response

compared to the other toxins (Crossley & Archer, 1997). It has been associated with conditions such as septic shock and also with staphylococcal food poisoning, an intoxication caused by the release of toxins by the organism before ingestion (Crossley & Archer, 1997). On the other hand, SEG, SEI, SELM, SELN, SELO gene clusters are associated with colonization and not infection (Dauwalder et al., 2006).

About 99% of *S. aureus* produce  $\gamma$ -hemolysins but their roles in pathogenesis is not well understood. However, it has been proposed that they play a role in toxic shock syndrome pathogenesis together with the Toxic Shock Syndrome Toxin -1 (TSST-1) (Clyne et al., 1988). The locus expresses three proteins, namely, HlgA, HlgB and HlgC encoded by the genes *hlgA*, *hlgB* and *hlgC*. These proteins assemble to form membrane perforating complexes that are toxic to polymorphonuclear cells, monocytes as well as macrophages and causes lysis of red blood cells (Hetem et al., 2016).

## **2.7 Antimicrobial Resistance in *Staphylococcus aureus***

### **2.7.1 Methicillin Resistance in *Staphylococcus aureus***

Methicillin resistance is one of the most significant developments in the history of *S. aureus* evolution. The first drug developed to treat staphylococcal infections was penicillin in the 1940s by Alexander Fleming. It was thought to be one of the most noteworthy advancements in healthcare. However, a few years after its introduction, resistance was developed (Deurenberg & Stobberingh, 2008). This resistance was a result of the organism producing an enzyme known as beta-lactamase encoded by the *blaZ* gene that inactivated the drug and allowed cell wall synthesis to take place in the organism (Hetem et al., 2016). Methicillin, a synthetic penicillin whose structure could not be destroyed by the beta-lactamase enzyme was then introduced in the 1960s to treat staphylococcal infections caused by penicillin-resistant *S. aureus* strains (Reygaert, 2013). Unfortunately, resistance was also developed to methicillin and down to this day, these strains are resistant to all beta-lactam ( $\beta$ -lactam) antibiotics such



as penicillin, cefoxitin, oxacillin and ampicillin as well as to other groups of antibiotics including macrolides and aminoglycosides (Chambers, 1997; Chambers & DeLeo, 2009). Although methicillin is no more in use and cefoxitin or oxacillin is used for phenotypic testing, the term MRSA still exists and is used to refer to *S. aureus* strains resistant to cefoxitin or oxacillin and a wide range of antibiotics. Methicillin resistance is understood to occur when Methicillin-Susceptible *S. aureus* (MSSA) acquires a gene known as *mecA* by gene transfer. This transfer is facilitated by a mobile genetic element called staphylococcal cassette chromosome mec (SCCmec). The *mecA* gene codes for a novel penicillin-binding protein PBP2a (Foster, 2004; Hetem et al., 2016). Penicillin-binding protein (PBP) is an essential enzyme that catalyzes peptidoglycan production in the cell wall of the bacteria (Farrington, 2012). All penicillin act by inhibiting the action of this enzyme causing an eventual destruction of the organism (Farrington, 2012). PBP2a, an altered form of PBP has low affinity for beta-lactam, thus, it prevents the beta-lactam ring of the drug from binding to it. This results in bacterial cell wall synthesis even when the antibiotic is present and bacterial survival (ECDC, 2015; Hetem et al., 2016). A divergent homologue of the *mecA* gene, *mecC* is also believed to confer resistance to methicillin (ECDC, 2015; Hetem et al., 2016). However, *mecC* codes for a different PBP and is usually found in strains from livestock (Hetem et al., 2016).

Since the late 1960s MRSA has been a major cause of healthcare-associated infections and at present is the most widespread resistant pathogen within the hospital (DeLeo et al., 2010). MRSA has spread worldwide with varying geographic prevalence (Moellering, 2012). In Europe for instance, there are reported variations in MRSA occurrences such as 0.9% in the Netherlands and 22.5% in Denmark (ECDC, 2015). However, MRSA prevalence has been reported to have decreased from 18.6% to 17.4% between 2011 and 2014 (ECDC, 2015). The Netherlands and Scandinavian countries particularly, have successfully reduced MRSA probably because of the ‘search and destroy’ policy put in place as well as the implementation

of effective infection control measures and restrictive antibiotic use (Hetem et al., 2016; Johnson, 2011). A decline is also occurring in the healthcare settings in the US. It has been reported that between 2005 and 2011, rates of invasive MRSA dropped by 31% (CDC, 2013). In Africa, MRSA isolation rates from 10.5 to 85.5% have been documented among patients with SSI (Ahmed, 2012; Dessie et al., 2016; Kahsay et al., 2014; Seni et al., 2013). Elsewhere such as India, Iran, Japan and China, rates between 30% and 53% have been reported (Adwan et al., 2016; Gu et al., 2015; Sasikumari et al., 2016; Takesue et al., 2017). In Ghana, attention has now been given to MRSA following outbreaks between 2012 and 2015 in some hospitals (Amissah et al., 2017; Donkor et al., 2018, 2019). Currently, routine detection of MRSA is being conducted in hospitals which was not the case in the past. This probably has contributed to surveillance in the country. MRSA prevalence has been reported to be generally low in Ghana (Donkor et al., 2019; Egyir et al., 2013). Documented prevalence is between 0.3% and 34.8% among nasal carriers and patients with infections (Amissah et al., 2015; Dekker et al., 2016; Egyir et al., 2013; Egyir et al., 2014; Eibach et al., 2017; Odonkor et al., 2012).

Since MRSA is resistant to both  $\beta$ -lactam and non  $\beta$ -lactam antibiotics, it is considered a threat and poses a real challenge in clinical practice, especially, in developing countries where antibiotic use is not regulated. There is also the issue of resistant gene transfer to other organisms. According to the CDC (2013), about 80,461 invasive MRSA infections occurred in 2011 out of which there were 11,285 deaths. Additionally, the European Centre for Disease Prevention and Control (ECDC) reported that, annually, 171,200 nosocomial infections are associated with MRSA among people in the EU member states, Iceland and Norway. This results in an excess hospital cost of €380 million and 5,400 deaths (ECDC, 2009).

Although MRSA has often been associated with healthcare settings, MRSA has emerged in the community among people without any prior exposure to the healthcare setting (Crossley et al., 2009; David & Daum, 2010; DeLeo et al., 2010). This strain over time is also showing

resistance to numerous antibiotics and moving from the community into healthcare facilities (Song et al., 2011) with one reported outbreak occurring in a maternal unit and nursery of a US hospital (Bratu et al., 2005). These strains are now even more predominant than healthcare-associated infections. Both strains have been shown to have the same virulence factors but community acquired strains usually carry a toxin known as Panton Valentine Leukocidin (PVL) (Reygaert, 2013). Also, community-associated MRSA tend to be susceptible to other classes of antimicrobials apart from  $\beta$ -lactams (David & Daum, 2010) and causes more of skin and soft tissue infections (Müller-Premru et al., 2005). Within the community, infections have also been associated with MRSA from livestock. These infections are believed to be transferred from individuals working with livestock (Cuny et al., 2015). MRSA strains are thus classified as healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA), depending on whether they are associated with hospitals, communities, or livestock (David & Daum, 2010; Gorwitz, 2008; Moellering, 2012).

### **2.7.2 Resistance Patterns of *S. aureus* and MRSA**

Antimicrobials have proven to be useful for the treatment of bacterial infections. However, the appearance of resistance has been witnessed in almost all pathogenic bacteria and poses a threat (ECDC, 2009). The degree of resistance to commonly used antibiotics varies from country to country (ECDC, 2015). This is influenced by the antibiotic use and misuse leading to selective pressure (ECDC, 2015). There are a number of antimicrobial drugs used to treat staphylococcal infections. These include clindamycin, erythromycin, tetracycline, flucloxacillin, cotrimoxazole, linezolid, daptomycin and vancomycin (Naik & Deshpande, 2011). The main drugs in Ghana are clindamycin and flucloxacillin.

In India, Kownhar et al. (2008) identified that *S. aureus* isolates from SSIs were highly resistant to gentamicin, penicillin and erythromycin, ampicillin and cefalexin. Similarly,

Bhave et al. (2016) found high resistance to penicillin, ciprofloxacin, amoxicillin and erythromycin. A study in Shanghai also detected high resistance to almost all antibiotics tested especially penicillin, gentamicin, erythromycin, tetracycline and clindamycin (Gu et al., 2015).

Across the African continent, resistance patterns of *S. aureus* to various drugs have been documented in many studies (Egyir et al., 2013; Feglo, & Afriyie-Asante, 2014; Kahsay et al., 2014; Seni et al., 2013). High resistance to penicillin, tetracycline and erythromycin is a prominent feature of *S. aureus* strains in Africa (Schaumburg et al., 2014). This, however, may not always be the case as observed in a remote area in central Gabon where majority of the *S. aureus* isolates were susceptible to penicillin and showed a low resistance rate to commonly used drugs such as tetracycline and co-trimoxazole (Schaumburg et al., 2011). This is the reverse for the study by Kahsay et al. (2014) in Ethiopia where a high proportion of *S. aureus* isolates were found to be resistant to erythromycin, co-trimoxazole, penicillin, gentamicin, and amoxicillin while all MRSA isolates detected were 100% resistant to co-trimoxazole and penicillin. This could infer that the resistance to commonly used antibiotics is higher in urban areas than in rural areas. Kesah et al. (2003) also published a study done between 1996 and 1997 in Malta and some African hospitals where MRSA isolates were sensitive to ciprofloxacin, rifampin and fusidic acid while majority were multi-drug resistant. In recent times, Seni et al. (2013) in Uganda also detected that MRSA isolates were sensitive to vancomycin and highly resistant to ampicillin and co-trimoxazole.

Most *S. aureus* strains circulating in Ghana have shown high resistance to penicillin and tetracycline (Amissah et al., 2015; Egyir et al., 2013; Egyir et al., 2014). In a rural area in the Ashanti region of Ghana, strains with high resistance to antibiotics frequently used by clinicians have been found. These isolates showed resistance particularly to penicillin, tetracycline and trimethoprim/sulfamethoxazole (co-trimoxazole) (Dekker et al., 2016). This

is a cause for alarm. However, MRSA strains have not been detected in high numbers in these areas and these isolated strains are sensitive to other drug used to treat staphylococcal infections (Dekker et al., 2016). Similarly, *S. aureus* from surgical wounds in the Ashanti region has been observed to be resistant to penicillin, ampicillin and erythromycin (Feglo & Afriyie-Asante, 2014). Conversely, in the Greater-Accra region, MRSA isolates from microbiological specimens have been reported with high resistance to penicillin and tetracycline as well as to ampicillin and flucloxacillin (Odonkor et al., 2012).

Vancomycin has been the last resort for treating MRSA infections (Gu et al., 2015). However, vancomycin-resistant and vancomycin-intermediate strains have been found (Gardete & Tomasz, 2014; Kheder et al., 2012). This makes serious infections caused by MRSA even more difficult to treat because there are a limited number of antimicrobial agents that could be used. Most of the studies mentioned above have documented sensitivity to vancomycin. In Ghana, high susceptibility to vancomycin may be attributable to the fact that these drugs are mostly unavailable and as such not frequently used (Labi et al., 2018).

## **2.8 Typing Methods for *S. aureus***

Typing is an essential tool for studying genetic diversity and determining the spread of organisms in order to develop effective surveillance systems for control (Deurenberg & Stobberingh, 2008). Many typing methods can be used to distinguish bacterial strains based on the genotypic characteristics and the occurrence of specific genes or genetic elements. These methods may be band or sequence-based. For *S. aureus*, the current methods used are Pulsed Field Gel Electrophoresis (PFGE), Staphylococcal Chromosome Cassette mec (SCCmec) typing, Staphylococcus Protein A (*spa*) typing and Multilocus Sequence Typing (MLST). These methods are widely used and vary in reproducibility, speed, ease discriminatory capacity as well as cost (Deurenberg & Stobberingh, 2008; Hetem et al., 2016). However, the type of method to use depends on the problem to be addressed (O'Hara et al.,

2016; Sabat et al., 2013). Methods with high discriminatory power are best used when characterizing for epidemiological investigation (O'Hara et al., 2016; Rodriguez et al., 2015).

### **2.8.1 Pulsed Field Gel Electrophoresis (PFGE)**

PFGE has been considered the gold standard and replaced phage typing. It is used widely for investigation of outbreaks and local epidemiological studies (Gu et al., 2015). This method however, is limited by the fact that it is time-consuming, comparing results from different laboratories is difficult, requires specialized equipment and is more expensive (Hetem et al., 2016). PFGE has gradually been replaced by MLST and *spa* typing.

### **2.8.2 *Spa* Typing**

This is a single-locus typing method based on variations in the sequences at the polymorphic X region of the *spa* gene which encodes Staphylococcus Protein A (Chambers & DeLeo, 2009). The X-region is made up of variable numbers of 24bp repeat sequences. Each 24bp repeat sequence is assigned a unique code. A combination of the different repeats gives the repeat succession of each isolate and determines its *spa* type. Diversity in the *spa* gene is often attributed to deletions, point mutations and duplications of the repeats (Chambers & DeLeo, 2009; Deurenberg & Stobberingh, 2008). This method has been used to study *S. aureus* and MRSA outbreaks as well as their molecular evolution (Chambers & DeLeo, 2009).

One advantage of this method is the availability of a software tool known as Ridom StaphTyper that provides easy sequence analysis. This software helps in *spa* type determination and provides a universal *spa* server database (<http://spaserver.ridom.de>) that allows for comparison of data across laboratories (Deurenberg & Stobberingh, 2008). Recently, a free accessible tool on the Centre for genomic epidemiology web page (<http://www.genomicepidemiology.org/>) has also been developed. Other advantages of this method over the others are that it is reproducible, easy to interpret and less expensive since it

involves only a single locus (Hetem et al., 2016). Some though have questioned this method because of the use of a single locus.

### **2.8.3 Multi-Locus Sequence Typing (MLST)**

MLST has been described as a great tool for studying the long periods of evolution in *S. aureus* (Chambers & DeLeo, 2009). This method is based on variations in the sequences of seven house-keeping genes *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL* (Deurenberg & Stobberingh, 2008). These genes are essential for bacterial survival thus present in all *S. aureus*. Each variation in the sequence of the gene is assigned a number, yielding a 7-numbered code that is specific for each sequence type (ST) and bacterial isolate. Since the housekeeping genes are not subject to a lot of change and selective forces, they provide more reliable information. However, this method is considered not suitable for routine infection control anymore as it is more expensive, time consuming and laborious (Strommenger et al., 2006). Yet, it is easy to compare or exchange the data generated internationally (Hetem et al., 2016). MLST and *spa* typing are most often used in combination with software-based clustering algorithms like BURST (Based Upon Related Sequence Types) and BURP (Based Upon Repeat Pattern) to place related isolates into clonal complexes (CCs) (O'Hara et al., 2016).

### **2.8.4 SCCmec Typing**

SCCmec typing is currently the primary typing method for MRSA characterization. This is a PCR based method used to determine the structures of the mobile genetic element SCCmec on which the *mecA* gene is borne (Deurenberg & Stobberingh, 2008). There are three protocols by which the SCCmec types are identified (Deurenberg & Stobberingh, 2008).

### **2.8.5 Whole Genome Sequencing (WGS)**

Whole genome sequencing is not a widely used tool for molecular characterization of bacterial strains because of its cost. However, recent advances in sequencing have made it more affordable and accessible. This tool allows for detailed analysis of entire genomes of

organisms (Kwong et al., 2015). WGS resulted from a revolution in sequencing technologies following the Human Genome Project. Advances were initially focused on improving Sanger sequencing developed in 1977 by Sanger and others. However, more efficient tools were later sort after which led to the establishment of ‘Shotgun sequencing’ (Kwong et al., 2015). However, this was laborious for sequencing whole genomes. Later advances in this technology led to the development of ‘whole-genome shotgun sequencing’ (Kwong et al., 2015). In recent times, sequencing technologies such as Next-Generation Sequencing (NGS) with easy and efficient library preparation protocols as well as simple benchtop systems have been developed (Kwong et al., 2015). This has made WGS more affordable, efficient and accessible for clinical and research applications (Kwong et al., 2015).

NGS methods generally have three steps: library preparation, amplification and sequencing. A number of library preparation kits are available that offer different protocols for sequencing both bacterial and viral pathogens, however, sequencing principles remain the same (Illumina, 2015). As opposed to Sanger sequencing which typically produces longer reads, NGS produces millions of small fragments of DNA in parallel (Behjati & Tarpey, 2013). The Roche/454, ABI/SOLiD, and Solexa/Illumina are the main NGS sequencing platforms, however, the Illumina is by far the most widely used technology (Hodzic et al., 2017). An additional and important part of sequencing is data analysis. Post-sequence data analysis can be done with commercially available or open source tools some of which have been developed for people with limited bioinformatics (Kwong et al., 2015). Analysis basically involves piecing together the fragments and mapping them to a reference genome from which a numerous analyses are possible (Behjati & Tarpey, 2013; Illumina, 2015).

There are a number of applications of WGS currently, which include identification of organisms, typing of bacterial strains, and detection of resistance and virulence factors (Kwong et al., 2015). Documented studies have used WGS to investigate outbreaks (Harris et



al., 2013; Kong et al., 2016), predict antimicrobial resistance (Gordon et al., 2014), type bacterial strains (Salipante et al., 2015) and to describe the transmission of MRSA (Harris et al., 2013).

Using this tool is considered better than other methods for typing because it gives a better resolution of bacterial isolates (Salipante et al., 2015). It also outperforms PFGE when it comes to discriminatory power and reproducibility (Salipante et al., 2015). WGS is however limited by the fact that analyses will depend on the quality of the genome assembly or sequencing and also on the quality and selection of the reference genome (Kwong et al., 2015). In addition, even though WGS data provides an extensive genomic information, it does not necessarily provide information about gene expression or transcription (Kwong et al., 2015). It has been proposed that other known methods of typing will probably be replaced by WGS as it represents the acme for characterisation of strains and epidemiological analyses (Kwong et al., 2015).

## **2.9 Molecular Epidemiology and Genetic diversity of *Staphylococcus aureus***

MRSA strains are biologically diverse and have been shown to vary from one geographic area to the other (Gorwitz, 2008) with some strains becoming dominant within certain areas and others globally distributed. In Eastern Australia, the common sequence type is ST30 while in Europe the major sequence type is ST80, and in Taiwan ST59 (Chambers & DeLeo, 2009). Even so, in Singapore all these strains have been reported possibly because there are millions of people visiting every year as it serves as an international travel hub (Hsu et al., 2005). Asadollahi et al. (2018) conducted a review to determine the predominant *spa* types in the world among clinical isolates. Results of the study indicate that the most prevailing *spa* type in Europe mainly UK and Germany is t032 followed by t008. This *spa* type t008 was also reported as the most prevalent in the USA and Canada. In Asia, the predominant *spa* type was

t030 followed by t037. *Spa* type t030 was also reported as the fifth most common type in Iran. In America, t002 was reported as the second most common *spa* type. This review also identified that there were sustained associations between *spa* type and sequence types. ST22 always associated with t032 regardless of the continent while ST8 and ST247 associated with t008. Some associated types were detected including: t002 with ST5, t030 with ST239, also ST22 and t037 with ST239. Another research aimed at determining the distribution of clones causing *S. aureus* invasive infections in Europe found that MSSA was more diverse than MRSA and among MSSA, the strains t002 (ST5) and t084 (ST15/18) were the most predominant. On the other hand, t032 (ST22) was predominant among MRSA isolates (Grundmann et al., 2010).

The PVL toxin is an important virulence factor and its carriage has been closely linked to infections caused by CA-MRSA (David & Daum, 2010). In Europe, PVL has been associated with CA-MRSA with a prevalence of less than 2% while in North America the prevalence is high (Rasigade et al., 2014). Contradictory to this is PVL-positive strains found on the African continent. These have been detected in high numbers with prevalence ranging from 17% to 74% and most often associated with MSSA. (Breurec et al., 2011; Egyir et al., 2014; Rasigade et al., 2014). It has been suggested that PVL-negative ST5 which is a paediatric clone and ST22 known as Barnim epidemic strain/EMRSA-15 were widespread for many years before PVL-positive ST5 and PVL-positive ST22 existed in Slovenia and Bavaria respectively (Linde et al., 2005; Müller-Premru et al., 2005). This worldwide epidemic strain ST5 was detected in skin and soft tissue infections associated with *spa* type t002 and PVL (Müller-Premru et al., 2005). This was the first study reporting PVL in ST5 in Europe.

ST152 and ST88 are strains often isolated from patients with a history of travel. ST152 has been identified as a pandemic clone in Macedonia and also in Slovenia (Monecke et al., 2007; Müller-Premru et al., 2005) suggesting that they are mainly distributed in Balkans (Monecke

et al., 2007). In Slovenia, it was detected in a team of footballers with severe skin and soft tissue infections caused by CA-MRSA. It should be noted that these footballers had no exposure to the healthcare setting (Müller-Premru et al., 2005). This pandemic clone has also been associated with PVL-positive CA-MRSA in Central Europe, the Balkan and Switzerland (Monecke et al., 2007; Ruimy et al., 2008).

In Middle Eastern countries including Gaza, CA-MRSA strains are mostly PVL-negative ST22. However, in Egypt and Jordan, PVL-positive ST80 known as the European clone is causing the CA-MRSA epidemic found there (Rasigade et al., 2014).

### **2.9.1 Molecular Epidemiology and Genetic Diversity of *Staphylococcus aureus* in Africa**

It is not until recently that efforts have been made to describe the molecular epidemiology of *S. aureus* strains in Africa. The majority of African countries including Ghana are widely dominated by *spa* type t084 and t355 (Amissah et al., 2015; Breurec et al., 2011; Dekker et al., 2016; Egyir et al., 2014; Schaumburg et al., 2014). ST239/241-MRSA-I/III/IV which is a Hungarian/ Brazilian clone is also prevailing in the African continent (Schaumburg et al., 2014). Other clones such as the well-known ST239/241-III pandemic clone (Deurenberg & Stobberingh, 2008) has been found among isolates from a multi-centre study across five towns in Madagascar, Morocco, Niger, Cameroon and Senegal (Breurec et al., 2011). ST88-MRSA-III/IV occasionally found in certain countries except Far East Asia also seems to be linked with Africa and has also been reported in Madagascar (Breurec et al., 2011; Schaumburg et al., 2014). Likewise, ST80-MRSA-IV a PVL- positive CA-MRSA clone found mainly in Europe but rarely found in sub-Saharan Africa is predominant in Maghreb (Schaumburg et al., 2014).

In addition, ST5-MSSA and ST5-MRSA have been observed to be widespread in Central and West Africa (Schaumburg et al., 2014). Schaumburg et al., (2014) further suggested that

considering that both strains are found in the same geographic area, ST5-MRSA may have evolved from ST5-MSSA acquiring SCCmec. However, this may not be the case for ST8MRSA and ST8-MSSA since they have different geographic locations. ST8-MSSA from Maghreb and ST8-MRSA from Central and West Africa.

Some publications have appeared in recent years documenting the diversity of *S. aureus* in Ghana. The prevalent *spa* types in Ghana are t355 and t084 (Amissah et al., 2015; Egyir et al., 2014; Eibach et al., 2017). Other *spa* types that have also been detected include: t314 and t311 (Amissah et al., 2015, 2017; Dekker et al., 2016; Donkor et al., 2019; Egyir et al., 2014; Eibach et al., 2017). The common pandemic clone ST152 has also been identified in Ghana as predominant, with the above mentioned studies documenting this as well as that of Kpeli et al. (2016). Many other sequence types are found to be abundant such as ST15, ST121 and ST5 associated with MSSA (Dekker et al., 2016; Egyir et al., 2014). Donkor et al. (2018) reported ST15 as a possible cause of the outbreak at the paediatric emergency ward of Korle-Bu Teaching Hospital. A study by Amissah et al. (2017) also reported high numbers of MRSA belonging to ST250 in a burn unit at KBTH although this was not PVL- positive. In this same study, four new *spa* types were identified: ST3248, ST3249, ST3250 and ST3251. These *S. aureus* isolates have also been associated with high PVL positivity which buttresses the point that Africa is a PVL endemic region (Schaumburg et al., 2014).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study Design**

This was a hospital-based cross-sectional study conducted over a 6-month period from June to November 2018 as part of a larger study entitled “Prevalence of MRSA/ESBL Producing Bacteria Associated with Surgical Site Infections in a Military and Civilian Hospital in Ghana” at Noguchi Memorial Institute for Medical Research (NMIMR). Patients seeking healthcare who had undergone surgery with infections at the site of incision were recruited into the study at the selected departments of the Korle-Bu Teaching Hospital and 37-Military hospital.

#### **3.2 Study Area**

The study was conducted at the 37-Military Hospital and Korle-Bu Teaching Hospital (KBTH) in Accra.

##### **3.2.1 37-Military Hospital**

The 37-Military hospital is the largest military hospital and also a specialist hospital located in Accra, Ghana. It has about 400 beds, a twenty four (24 hr) accident and emergency department and pharmacy (“The Electives Network: 37 Military Hospital,” n.d.). It caters for both military and civilian patients as such, it sees a diverse number of patients. This site was included in the study because it also serves as a referral hospital and thus, receives referral cases. Additionally, the funding body [Global Emerging Infectious Surveillance (GEIS)] supports mainly military surveillance research.

The study was conducted at the following departments in the hospital: surgical OPD, Ghandi ward, Tamakloe ward, trauma and surgical emergency unit and orthopaedic ward. The surgical OPD sees patients who had surgeries and have been discharged from the hospital. These patients return to the hospital about twice a week for wound dressings and may be recruited

into the study. The Ghandi ward is recovery ward for female patients after surgery while the Tamakloe ward is a recovery ward of both male and female surgical patients. On the other hand, the trauma and surgical emergency unit is the first point of call for patients involved in accidents. A few of these patients who undergo surgery and are discharged often report to the unit when they have infections.

### **3.2.2 Korle-Bu Teaching Hospital**

Korle-Bu teaching hospital is the largest hospital and leading referral hospital in Ghana. It has a hospital bed capacity of 2000 as well as 17 clinical and diagnostic departments which comprise obstetrics and gynaecology, surgical/medical emergency, surgery, and an accident centre (“About us – Brief History – Korle-Bu Teaching Hospital,” n.d.). An average of 1,500 people visit the hospital each day and there are about 250 admissions (“About us – Brief History – Korle-Bu Teaching Hospital,” n.d.). Apart from being a referral hospital, it is also a teaching hospital which trains medical doctors and a wide range of health professionals. There is also a 42-bed polyclinic that provides care for residents in the community as well as other parts of Accra (“About us – Brief History – Korle-Bu Teaching Hospital,” n.d.). Initially, it was set-up to be a service delivery facility within the community but has developed beyond that. This site was included in the study because it receives lot of referral cases from other regions of the country in addition to referrals from all parts of the city. Most importantly, this is the hospital where most complicated surgeries are performed.

Patients recruited from this health care facility were from the following departments: obstetrics and gynaecology, surgical, orthopaedics, maternity, neurosurgery and paediatrics.

### **3.3 Ethical Clearance**

Ethical approval was obtained from the Noguchi Memorial Institute Institutional Review Board, Korle-Bu Institutional Review Board, 37-Military Hospital Institutional Review Board and the Naval Medical Research Centre Institutional Review Board.

Approvals were also sought from the various departments of the Korle-Bu Teaching Hospital and the 37-Military hospital where the study was conducted.

### **3.4 Recruitment of Study Participants**

Out-patients reporting to the hospital for wound dressing after surgery, as well as in-patients who had undergone surgery with infections and fit the eligibility criteria were recruited into the study. Participants were either given consent forms to read and sign or had the consent forms read and explained to them, then the forms were signed in the presence of a witness. All participants below the age of eighteen were given child assent forms and parental consent was also sought from their guardians. Participants were then given a study ID to maintain confidentiality. The field staff completed the case report forms using the patient folder information and patient's answers. Demographic data such as age, sex, as well as type of surgical operation, type of infection, co-morbidities, period of hospitalization and antimicrobial therapy were also collected.

#### **3.4.1 Inclusion Criteria**

Selected participants comprised patients who had undergone surgery and had developed infections with purulent discharge at the site of incision. The infection should have been diagnosed by a physician and met the CDC criteria for classification as a surgical site infection (CDC, 2015; Mangram et al., 1999).

##### **3.4.1.1 CDC Criteria**

The CDC requires that for a patient to meet the criteria, the patient should have had a National Healthcare Safety Network (NHSN) operative procedure. NHSN defines an operative procedure as one that takes in an operating room where at least one incision is made through the skin or mucous membrane or reoperation through an incision that was left open during a prior operative procedure. The period for infection should be 30 or 90 days after surgery depending on the procedure performed and the depth of the infection (CDC, 2015; Mangram

et al., 1999). NHSN provides a list of operative procedures that should be followed for 30 or 90 days. This table can be found in Appendix IX.

### **3.4.2 Exclusion Criteria**

Patients who had undergone surgery, had developed infections but not within the CDC classification as a surgical site infection were excluded.

## **3.5 Sample Collection**

A trained study nurse or a clinician aseptically collected either a wound swab, fluid or aspirate from each patient using either a sterile cotton-tipped applicator or a syringe respectively. The wound was first cleaned with normal saline to reduce the skin flora before the samples were taken. For wound swabs, a part of the handle of the applicator stick was broken off and the rest of the stick was placed in a 15ml falcon tube containing 10ml of thioglycollate broth. Aspirates and fluids were dispensed into the tube with the thioglycollate broth. The samples were placed in an ice chest and transported at room temperature to NMIMR within 24 hours to maximize the chance of isolating the causative organism.

## **3.6 Laboratory Procedures**

### **3.6.1 Sample Processing**

Upon arrival at the Naval Medical Research Unit three (NAMRU-3) laboratory at NMIMR, the sample IDs were entered into a sample receiving book and inspected to make sure the right samples were collected. The samples were then incubated aerobically at 37°C for 18-24 hours. After incubation, the samples were cultured on mannitol salt agar, blood agar and then incubated aerobically for another 18-24 hours.

### **3.6.2 Identification of Bacterial Isolates**

*Staphylococcus spp* isolates were identified using colonial morphology, Gram stain characteristics, catalase test and coagulase test. Using colonial morphology, *Staphylococcus*



*spp* were identified by 2mm white or cream-coloured to golden beta-hemolytic colonies on blood agar plate, and 2-3mm yellow colonies on mannitol salt agar plate (Forbes et al., 2007; Maza et al., 2004).

#### **3.6.2.1 Gram Stain**

Using a sterile 1mm loop, one to two colonies of freshly cultured bacteria were placed on a clean slide containing a drop of saline. The colonies were emulsified in the saline and allowed to air-dry. All slides were heat fixed prior to Gram staining. Gram stain was performed using the BD BBL Gram Stain kit (Becton Dickinson, Australia). Staining was done by adding crystal violet for one minute after which the slide was washed with water. Subsequently, the slide was flooded with iodine for another minute and washed off. The slide was then decolourized with a decolourizer then immediately washed off with water. Finally, Safranin was added for another minute and washed off. The slides were allowed to air-dry and viewed under the oil immersion lens (100X) of the microscope. Gram-positive cocci in clusters were observed.

#### **3.6.2.2 Catalase Testing**

The catalase test was performed using a catalase reagent dropper (Becton Dickinson, Australia). A drop of the reagent was placed on a clean slide and a colony of the bacteria was placed in the reagent. A catalase-positive test was observed as a fizzing reaction. This indicated a conversion of hydrogen peroxide to water and oxygen.

#### **3.6.2.3 Coagulase Testing**

A coagulase test was then used to differentiate *S. aureus* isolates from other staphylococci.

Coagulase testing was performed using the BD BBL Coagulase rabbit plasma (Becton Dickinson, Australia). One millilitre (1ml) of rabbit plasma was placed in a sterile tube and a few colonies of the test bacteria were added to it. The tubes were incubated aerobically at 37°C

for four hours. A coagulase-positive reaction was observed by formation of a clot. For isolates that showed negative after four hours, they were re-incubated for up to 24 hours to confirm they were negative. A coagulase-positive standard strain *Staphylococcus aureus* ATCC 25923 and a coagulase-negative standard strain *Staphylococcus epidermidis* ATCC 12228 was used as a quality control.

### **3.6.3 Confirmation of Isolates Identified**

All *Staphylococcus spp* isolates were confirmed using the Matrix Assisted Laser Desorption/Ionization (MALDI) Biotyper (Bruker, USA).

The MALDI machine has three main components which are the ionization source, the analyser and detector as well as an inlet for sample loading. Once the sample is loaded, it is ionized by matrix-assisted laser desorption ionization (MALDI) method. The machine then uses a database of known organism to match the isolate on the target plate while providing a matching score. Scores >2 are considered as very good matches.

Samples were prepared by placing 1-2 colonies of *Staphylococcus spp* on a stainless-steel plate known as the target plate using a 1µ loop and allowed to air-dry. One microliter (1µl) of formic acid was then added and allowed to air-dry. Finally one microliter (1µl) of matrix was added, allowed to air-dry and the target plate was then placed in the machine. The matrix is an organic, energy-absorbent compound which facilitates the ionization process. It works to absorb energy in the form of ultraviolet light and transfers to the larger sample molecules.

Once inside the MALDI machine, the target is brought to a vacuum and struck by a pulsed ultraviolet laser. The matrix absorbs the laser energy and then it is gradually removed from the surface of the sample carrying along the analyte molecules of the sample with it into a gaseous phase. The process of removal is known as ablation. During the ablation process, the analyte molecules are ionized by proton transfer from the nearby matrix molecules. The gas

phase analytes ions are then analysed by the Time of Flight (TOF) spectrometer. The TOF spectrometer accelerates the gas phase ions in a high voltage electric field which imparts a constant amount energy and this causes the smallest ions to travel the fastest. The TOF detector then records the time it takes for the different groups of ions to travel a certain distance. The spectrometer is then able to calculate and record the mass to charge ratio of each ion it detects. The TOF information is used to generate a characteristic spectrum for the analytes in the sample. This is known as the Peptide Mass Fingerprint (PMF). These peptides are proteins unique to the organism that are cleaved into smaller fragments. The microorganism is identified by comparing the PMF of the unknown organism with the PMF of the known organism in the systems database. This allows for identification of the microorganism to the genus and in many instances the species level (Singhal et al., 2003). It took just a few minutes for the machine to generate these results.

#### **3.6.4 Antimicrobial Susceptibility Testing**

Antimicrobial testing was performed using the Kirby-Bauer Disk diffusion method. An inoculum equivalent to 0.5 McFarland standard was prepared using physiological saline and the turbidity was measured with a Nephelometer. The inoculum was streaked on Mueller Hinton agar using a sterile cotton tipped applicator after which the antibiotic impregnated disks were placed on the agar aseptically and incubated aerobically at 37°C for 18-24hrs.

Antibiotics recommended by Clinical and Laboratory Standard Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for testing. The antibiotics tested included: Penicillin (1Unit), Cefoxitin (30µg), Tetracycline (30µg), Clindamycin (2µg), Erythromycin (15µg), Gentamicin (10µg), Rifampicin (5µg), Trimethoprim/Sulfamethoxazole (1.25/23.75µg), Linezolid (10µg), Norfloxacin (10µg) and Vancomycin E-test strips. The zones of inhibition were measured with a digital calliper and zone sizes interpreted using the CLSI (2018) guideline. For antibiotics [Penicillin (1Unit),

Linezolid (10µg), and Rifampicin (5µg)] whose concentrations could not be found in the CLSI guideline, the EUCAST 2018 guideline was used. All isolates with Cefoxitin zone sizes  $\leq 21$ mm were considered presumptive MRSA. The *S. aureus* standard strain ATCC 25923 was used as a quality control for all antimicrobial sensitivity testing.

### **3.7 Molecular Techniques**

#### **3.7.1 DNA Extraction**

Crude DNA from was extracted from *S. aureus* isolates as described by Dashti et al. (2009) with slight modifications. Briefly, 2-6 pure colonies from an overnight culture were suspended in a 1.5 ml Eppendorf tube containing two hundred microliters (200µl) of nuclease-free water. The tube was placed on a heating block set at 100°C and allowed to boil for 10 minutes. The suspension was then centrifuged for 5 minutes at a speed of 13,000rpm to separate the pellet and supernatant. Finally the supernatant was pipetted into a new 1.5 ml Eppendorf tube leaving behind the pellet. All extracts were stored at -20°C until further testing.

#### **3.7.2 Polymerase Chain Reaction**

A multiplex PCR was performed to detect the *spa*, *pvl* and *mecA* genes following the Larsen et al. (2008) protocol with slight modifications. A primer mix containing primers for all genes mentioned in Table 3.1 was prepared prior to master mix preparation. The concentration of each primer (forward and reverse) was as follows 0.18µM for *spa*, 1µM for *pvl* and 0.45µM for *mecA*. The primer sequences are listed in Table 3.1. To make a (1ml) primer mix containing the above concentrations, a volume of each 10µM forward and reverse primer was picked and an amount of water was added. The volumes for each primer are listed in Table 3.2. Each PCR tube contained a 25µl reaction volume consisting of 12.5µl Multiplex PCR Mastermix (Qiagen, Germany), 2.5µl RNase-free water (Qiagen, Germany), primer mix of 8µl and a DNA template of 2µl. DNA amplification was performed using an Eppendorf Mastercycler x50s with the following cycling conditions: initial denaturation at 94°C for

15mins, 30 cycles of 94°C for 30sec, 57°C for 1 min, 72°C for 1min followed by a final extension at 72°C for 10mins. A positive control showing all three genes and a negative control (nuclease-free water) were included to ensure the results were accurate.

**Table 3.1: Primer Sequences used in the Study**

| Primer      | Primer sequences                                                              | Description                                              | References            |
|-------------|-------------------------------------------------------------------------------|----------------------------------------------------------|-----------------------|
| <i>spa</i>  | F: 5'-TAAAGACGATCCTTCGGTGAGC-3'<br>R: 5'-CAGCAGTAGTGCCGTTTGCTT-3'             | To detect <i>spa</i> gene ( <i>S. aureus</i> specific)   | (Larsen et al., 2008) |
| <i>pvl</i>  | F: 5'-GCTGGACAAA<br>ACTTCTTGGAATAT-3'<br>R: 5'-GATAGGACACCAATAAATTCTGGATTG-3' | To detect Panton Valentine Leukocidin (virulence factor) | (Larsen et al., 2008) |
| <i>mecA</i> | F: 5'-TCCAGATTACA ACTTCACCAGG-3'<br>R: 5'-CCACTTCATATCTTGTAACG-3'             | To detect methicillin resistance                         | (Larsen et al., 2008) |

**Table 3.2: One millilitre (1ml) Primer mix preparation**

| Primer              | Forward       | Reverse |
|---------------------|---------------|---------|
| <i>mecA</i> (10uM)  | 45µl          | 45µl    |
| <i>spa</i> (10uM)   | 18µl          | 18µl    |
| <i>pvl</i> (10uM)   | 100µl         | 100µl   |
| Nuclease free water | 674µl         |         |
| Total volume        | <b>1000µl</b> |         |

### 3.7.3 Gel Electrophoresis

A 2% w/v agarose gel was used to analyse the amplified products. The expected band sizes for the various genes were: *spa* (variable region- 200-600bp), *mecA* (162bp), and *pvl* (80bp)

### **3.7.3.1 Gel Preparation**

The gel was prepared by first weighing 1.5g of agarose powder and adding to 75 ml of Tris acetate-EDTA (TAE) buffer which had been measured into a beaker. The TAE buffer and agarose powder mixture was microwaved for at least 2 mins or until the mixture was clear. The resultant mixture was allowed to cool. SYBR Safe DNA gel stain (10,000X concentrate) (Invitrogen, USA) of 7.5µl was added and mixed thoroughly. The mixture was then poured into a casting tray with combs inserted and left for at least 30 mins to solidify after which the combs were removed.

### **3.7.3.2 Gel loading and Running**

The casting tray containing the gel was transferred into the gel tank and the tank was filled with 1X TAE Buffer. The wells were loaded with 5ul of a 100bp ladder, 7ul of each sample as well as a positive and negative control. A blue/orange 6x loading dye (Promega, USA) was used to load the PCR products. The gel was run at 80 Volts for at least one (1) hr. Subsequently, the gels were visualised using the LED Illuminator (BT Lab Systems) and photographed using the UVP BioDoc-It Imaging system (Analytik Jena, Germany).

### **3.7.4 DNA Extraction for Sequencing**

For sequencing purposes, DNA was re-extracted using the Lucigen extraction kit following the manufacturer's instructions with slight modifications. Here, the bacterial colonies were picked from the culture plate with an inoculation loop and suspended in 300µl of 1X Phosphate Buffered Saline (PBS). The suspension was vortexed and centrifuged at 10,000 rpm for 5 mins. Two hundred and seventy-five microliters of the supernatant was discarded leaving behind the pellet and approximately 25µl of the supernatant. The pellet was then resuspended by vortexing for 10 seconds. One microliter of Proteinase K was diluted into 300µl of tissue and cell lysis solution and added to each sample. The tube was vortexed to thoroughly mix its contents and incubated at 65°C for 30 minutes on a rocking platform. After

incubation, the samples were cooled to 37°C and 1µl of 5mg/ml RNase A was added. The mixture was incubated again at 37°C for 1 hour and then placed on ice for 3-5 minutes. Subsequently, 150µl of MPC Protein Precipitation reagent was added and vortexed for 10 seconds. The tube was then centrifuged at 10,000xg for 10 minutes at a temperature of 4°C. For pellets that were clear, small or loose, an additional 25µl of MPC Protein Precipitation reagent added and centrifuged again. The supernatant was transferred into a new 1.5ml tube and 500µl of isopropanol was added to it. Afterwards, the tube was inverted 30-40 times and centrifuged for 10 minutes at a temperature of 4°C. Without disturbing the pellet, the isopropanol was pipetted out and the pellet was rinsed twice with 70% ethanol by adding and pouring out while still ensuring the pellet is not lost. Finally, the pellet was resuspended in 35µl of TE buffer. DNA extracts were stored in -20°C until further use.

### **3.7.5 Whole Genome Sequencing**

Sequencing was done using the Nextera DNA Flex Library preparation kit and sequenced with the Mi-Seq Illumina sequencer. The library preparation was done according to the manufacturer's instruction.

The extracted DNA was quantified using a Qubit double strand (ds) high sensitivity (HS) kit (Thermo Fisher Scientific, USA). DNA concentration between 100 to 500ng in 30µl was used as starting material for library preparation. The DNA was tagmented using the Beaded-Linked Transposomes (BLT), which involved fragmenting and tagging the DNA with adapter sequences. This process was followed by a post tagmentation clean-up, which was done to stop tagmentation and wash the adapter-tagged DNA before amplification by Polymerase chain reaction (PCR). The PCR process then amplified the tagmented DNA and added paired index adapters and sequences required for cluster formation on the flow-cell during sequencing. Subsequently, a post PCR amplification clean-up was done to purify amplified libraries and remove excess unbound index adapters. The amplified libraries were quantified

with the 2100 bioanalyzer system (Agilent, USA) and subsequently by quantitative PCR (qPCR) using the Kapa Sybr Fast qPCR kit (Kapa Biosystems, USA). Using the fragment sizes generated by the bioanalyzer, and the concentration of individual libraries from the qPCR, the libraries were normalised, pooled and loaded onto the Mi-Seq sequencer (Illumina, USA).

The Mi-Seq sequencer generated 250 paired-end reads with barcoding. An in-house pipeline by US Army Medical Research Institute for Infectious Diseases (USAMRIID) was used to blast the reads to the NCBI non-redundant database. The best matching sequences were selected and used as references to perform reference based assembly. Assembly was done using an in-house pipeline which uses Bowtie to map the reads to a selected reference. These data files were then uploaded in the centre for genomic epidemiology webpage to determine the *spa* types, sequence types and toxin genes present.

### **3.8 Statistical Analysis**

Demographic data and laboratory results were entered into Access 2007 and exported to STATA software version 13 for analysis. Descriptive statistics was used to analyse data from laboratory findings and then presented using frequencies, percentages and bar graphs. Associations were determined using Fisher's exact test. P-values <0.05 were considered significant.



## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Demographic Characteristics of Study Participants

A total of 110 patients with surgical site infections were recruited from the two hospitals. Fifty-four of these patients were enrolled at the 37-Military Hospital; the remaining 56 patients were recruited from Korle-Bu Teaching Hospital.

Patients recruited at the 37-Military Hospital consisted of 27 (50%) males and 27 (50%) females. Their ages ranged from 5 years to  $\geq 65$  years. At Korle-Bu Teaching Hospital, 7 (12.5%) males and 49 (87.5%) females were enrolled. Their age range was  $<5$  years to 65 years. Patients between the ages of 25-44 years were the highest number enrolled at both sites. A summary of the demographic characteristics of study participants can be found in Table 4.1.

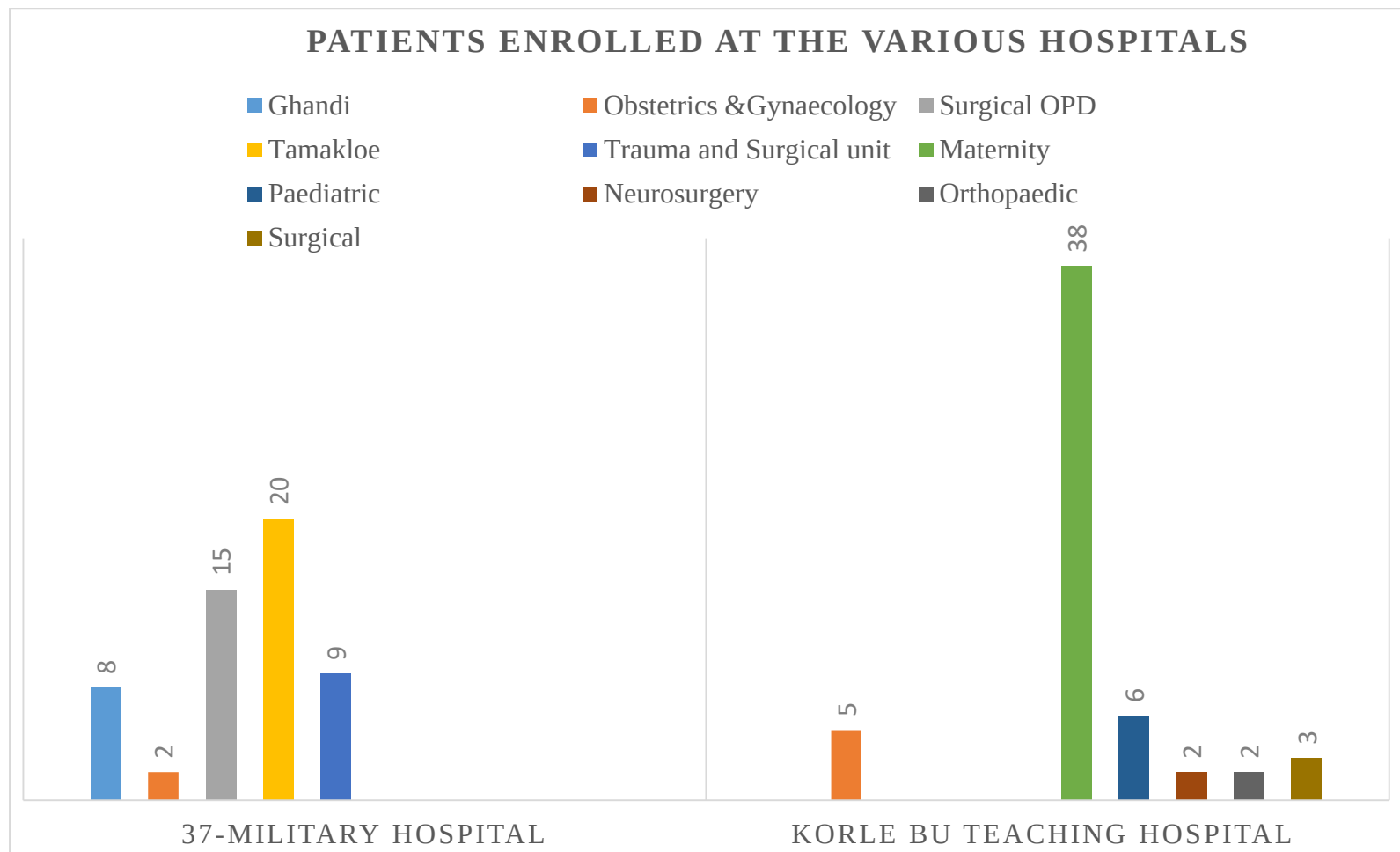
**Table 4.1: Demographic Characteristics of Study Participants**

| Variable                         | Total<br>N (%) | 37 Military<br>Hospital<br>n (%) | Korle Bu<br>Teaching<br>Hospital<br>n (%) | P-value |
|----------------------------------|----------------|----------------------------------|-------------------------------------------|---------|
| <b>Total</b>                     | 110            | 54                               | 56                                        |         |
| <b>Gender</b>                    |                |                                  |                                           |         |
| <b>Male</b>                      | 34 (12.5)      | 27(50.0)                         | 7(12.5)                                   | 0.000   |
| <b>Female</b>                    | 76 (69.1)      | 27(50.0)                         | 49 (87.5)                                 |         |
| <b>Age</b>                       |                |                                  |                                           |         |
| <b>&lt;5 years</b>               | 2(2.6)         | 0(0.0)                           | 2(6.3)                                    | 0.004   |
| <b>5-14 years</b>                | 2(2.6)         | 1(2.5)                           | 1(3.1)                                    |         |
| <b>15-24years</b>                | 10(13.0)       | 5(11.1)                          | 5(15.6)                                   |         |
| <b>25-44years</b>                | 38(49.4)       | 17(37.8)                         | 24(65.6)                                  |         |
| <b>45-64years</b>                | 2(20.8)        | 14(31.1)                         | 2(6.3)                                    |         |
| <b><math>\geq 65</math>years</b> | 9(11.7)        | 8 (17.8)                         | 1(3.1)                                    |         |

#### **4.2 Patients Enrolled at the Various Departments/Hospitals**

The highest number of patients enrolled at 37-Military Hospital were from the Tamakloe ward and surgical OPD. The maternity ward enrolled the highest number of patients at Korle-Bu Teaching Hospital. Figure 4.1 shows the distribution of patients at the departments.

**Figure 4.1: Distribution of Patients Enrolled into the Study**



### 4.3 Antibiotic Use before and after Operation

Data collected on patients' antibiotic use before and after operation indicated that, majority of the patients at 37-Military Hospital took Ciprofloxacin 13(24.07%) before operation and this was followed by clindamycin 12(22.22%). At Korle-Bu Teaching Hospital, most of the patients took Metronidazole 40(71.43%) before operation, followed by Amoxicillin-Clavulanic acid 33(58.93%). A summary of preoperative antibiotic use can be found in Table 4.2. The study recorded a post-operative use of metronidazole by 65 (59.09%) patients. Thirty-nine (72.22%) of those patients were from 37-Military Hospital while the remaining 26 (46.43%) patients were from Korle-Bu Teaching Hospital. The second most used antibiotic at both sites was Amoxicillin- Clavulanic acid 39(35.45%). A summary of post-operative antibiotic use can be found in Table 4.3.

**Table 4.2: Preoperative Antibiotic Use**

|                                    | <b>37 Military Hospital<br/>n (%)</b> | <b>Korle Bu Teaching<br/>Hospital n (%)</b> | <b>Total<br/>N (%)</b> |
|------------------------------------|---------------------------------------|---------------------------------------------|------------------------|
| <b>Total</b>                       | 54                                    | 56                                          | 110                    |
| <b>Antibiotic</b>                  |                                       |                                             |                        |
| <b>Metronidazole</b>               | 6(11.11)                              | 40(71.43)                                   | 46(41.82)              |
| <b>Amoxicillin-<br/>Clavulanic</b> | 7(12.96)                              | 33(58.93)                                   | 40(36.36)              |
| <b>Clindamycin</b>                 | 12(22.22)                             | 4(7.14)                                     | 16(14.55)              |
| <b>Ciprofloxacin</b>               | 13(24.07)                             | 2(3.57)                                     | 15(13.64)              |
| <b>Flucloxacillin</b>              | 0(0)                                  | 4(7.14)                                     | 4(3.64)                |
| <b>Gentamicin</b>                  | 1(1.85)                               | 3(5.36)                                     | 4(3.64)                |
| <b>Cefuroxime</b>                  | 2(3.70)                               | 2(3.57)                                     | 4(3.64)                |
| <b>Ceftriaxone</b>                 | 1(1.85)                               | 3(5.36)                                     | 4(3.64)                |
| <b>Meropenem</b>                   | 1(1.85)                               | 0(0)                                        | 1(0.91)                |
| <b>Flucomox</b>                    | 1(1.85)                               | 0(0)                                        | 1(0.91)                |
| <b>Cefotaxime</b>                  | 0                                     | 1(1.79)                                     | 1(0.91)                |

**Table 4.3: Post-Operative Antibiotic Use**

|                                    | <b>37 Military<br/>Hospital<br/>n (%)</b> | <b>Korle Bu Teaching<br/>Hospital<br/>n (%)</b> | <b>Total<br/>N (%)</b> |
|------------------------------------|-------------------------------------------|-------------------------------------------------|------------------------|
| <b>Total</b>                       | 54                                        | 56                                              | 110                    |
| <b>Antibiotic</b>                  |                                           |                                                 |                        |
| <b>Metronidazole</b>               | 39(72.22)                                 | 26(46.43)                                       | 65(59.09)              |
| <b>Amoxicillin-<br/>Clavulanic</b> | 26(48.15)                                 | 13(23.21)                                       | 39(35.45)              |
| <b>Clindamycin</b>                 | 9(16.67)                                  | 14(25)                                          | 23(20.9)               |
| <b>Ciprofloxacin</b>               | 2(3.70)                                   | 15(26.79)                                       | 17(15.45)              |
| <b>Cefuroxime</b>                  | 5(9.26)                                   | 8(14.29)                                        | 13(11.82)              |
| <b>Gentamicin</b>                  | 4(7.41)                                   | 2(3.57)                                         | 6(5.45)                |
| <b>Ceftriaxone</b>                 | 5(9.26)                                   | 0(0)                                            | 5(4.55)                |
| <b>Levofloxacin</b>                | 0(0)                                      | 2(3.57)                                         | 2(1.82)                |
| <b>Meropenem</b>                   | 1(1.85)                                   | 0(0)                                            | 1(0.91)                |
| <b>Amikacin</b>                    | 1(1.85)                                   | 0(0)                                            | 1(0.91)                |
| <b>Augmentin</b>                   | 0(0)                                      | 1(1.79)                                         | 1(0.91)                |
| <b>Flucloxacillin</b>              | 0(0)                                      | 1(1.79)                                         | 1(0.91)                |
| <b>Fluconazole</b>                 | 1(1.85)                                   | 0(0)                                            | 1(0.91)                |

#### 4.4 Proportion of *Staphylococcus aureus* Isolated from Study Participants

A total of 110 samples were collected for testing (Wound swabs= 106, Aspirate= 4). All 110 cultured samples showed growth. Some with polymicrobial growth and others with monomicrobial growth. Out of the 110 samples cultured, 13 (11.8%; 13/110) *Staphylococcus aureus* isolates were recovered. Eight (14.8%; 8/54) of the isolates were recovered from 37Military Hospital and five (8.9%; 5/56) from Korle-Bu Teaching Hospital. A summary of the various proportions can be found in Table 4.4.

**Table 4.4: Proportions of Patients Positive for *Staphylococcus aureus* at the two Hospitals**

| <b>37-Military Hospital<br/>N=54</b> | <b>Korle-Bu Teaching<br/>Hospital N= 56</b> | <b>Total<br/>N=110</b> |
|--------------------------------------|---------------------------------------------|------------------------|
| <b>n (%)</b>                         | <b>n (%)</b>                                | <b>N (%)</b>           |
| 8 (14.8)                             | 5 (8.9)                                     | 13 (11.8)              |

#### **4.5 Antimicrobial Resistance Pattern of *Staphylococcus aureus***

All the 13 (100%) *S. aureus* isolates showed resistance to penicillin. At 37-Military Hospital, 4 (50%) isolates were resistant to tetracycline. Resistance was recorded for all the other antibiotics except for gentamicin and vancomycin. At Korle-Bu Teaching Hospital, 2 (40%) of the 5 isolates were resistant to tetracycline while 1(20%) of the isolate was resistant to norfloxacin. There was no resistance recorded for ceftiofur, clindamycin, linezolid, Trimethoprim-Sulfamethoxazole (Co-trimoxazole), erythromycin, rifampicin, gentamicin and vancomycin. Table 4.5 shows the resistance profile of the isolates at both sites.

**Table 4.5: Antimicrobial Resistance Profile of *Staphylococcus aureus* Isolates**

| <b>Antibiotic</b> | <b>37-Military<br/>Hospital n (%)</b> | <b>Korle-Bu Teaching<br/>Hospital n (%)</b> | <b>Total no of<br/>Isolates<br/>N (%)</b> |
|-------------------|---------------------------------------|---------------------------------------------|-------------------------------------------|
| Penicillin        | 8 (61.5)                              | 5 (38.5)                                    | 13 (100)                                  |
| Tetracycline      | 4 (30.8)                              | 2(15.4)                                     | 6 (46.2)                                  |
| Cefoxitin         | 4 (30.8)                              | 0 (0)                                       | 4 (30.8)                                  |
| Clindamycin       | 2 (15.4)                              | 0 (0)                                       | 2 (15.4)                                  |
| Linezolid         | 5 (38.5)                              | 2 (15.4)                                    | 7 (54.0)                                  |
| Norfloxacin       | 1 (7.7)                               | 1 (7.7)                                     | 2 (15.4)                                  |
| Erythromycin      | 1 (7.7)                               | 0 (0)                                       | 1 (7.7)                                   |
| Rifampicin        | 1 (7.7)                               | 0 (0)                                       | 1 (7.7)                                   |
| Co-trimoxazole    | 1 (7.7)                               | 0 (0)                                       | 1 (7.7)                                   |
| Gentamicin        | 0 (0)                                 | 0 (0)                                       | 0 (0)                                     |
| Vancomycin        | 0 (0)                                 | 0 (0)                                       | 0 (0)                                     |

#### **4.6 Antimicrobial Resistance Profile of MRSA and MSSA**

A summary of the susceptibility patterns for the 13 isolates is shown in Table 4.6. MRSA isolates showed susceptibility to gentamicin, rifampicin and co-trimoxazole and vancomycin. Two of the 4 MRSA isolates showed reduced susceptibility to erythromycin. Three of the 4 isolates were also susceptible to linezolid and norfloxacin while 2 were susceptible to tetracycline and clindamycin. The highest resistance for MRSA and MSSA was recorded for penicillin 13(100%). MSSA showed a similar resistance pattern to MRSA with a few exceptions.

**Table 4.6: Antimicrobial susceptibility Patterns of MRSA and MSSA Isolates**

| MRSA           |     |     |     |     | MSSA |     |     |     |     |     |     |     |     |
|----------------|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|
| Antibiotic     | Is1 | Is2 | Is3 | Is4 | Is1  | Is2 | Is3 | Is4 | Is5 | Is6 | Is7 | Is8 | Is9 |
| Cefoxitin      | R   | R   | R   | R   | S    | S   | S   | S   | S   | S   | S   | S   | S   |
| Tetracycline   | R   | R   | S   | S   | S    | R   | R   | S   | R   | R   | S   | S   | S   |
| Penicillin     | R   | R   | R   | R   | R    | R   | R   | R   | R   | R   | R   | R   | R   |
| Clindamycin    | S   | I   | R   | S   | S    | R   | S   | S   | S   | S   | S   | S   | S   |
| Erythromycin   | S   | I   | R   | I   | S    | S   | I   | I   | I   | S   | S   | S   | S   |
| Gentamicin     | S   | S   | S   | S   | S    | S   | S   | S   | S   | S   | S   | S   | S   |
| Linezolid      | S   | R   | R   | S   | R    | S   | R   | R   | R   | R   | S   | S   | S   |
| Rifampicin     | S   | S   | S   | S   | S    | S   | R   | S   | S   | S   | S   | S   | S   |
| Norfloxacin    | S   | R   | S   | S   | S    | S   | I   | S   | S   | S   | S   | S   | R   |
| Co-trimoxazole | S   | S   | S   | S   | S    | S   | R   | S   | S   | S   | S   | S   | S   |
| Vancomycin     | S   | S   | S   | S   | NT   | NT  | NT  | NT  | NT  | NT  | NT  | NT  | NT  |

**Abbreviations: Is- Isolate, R- Resistant, S- Susceptible, I- Intermediate NT-Not tested**

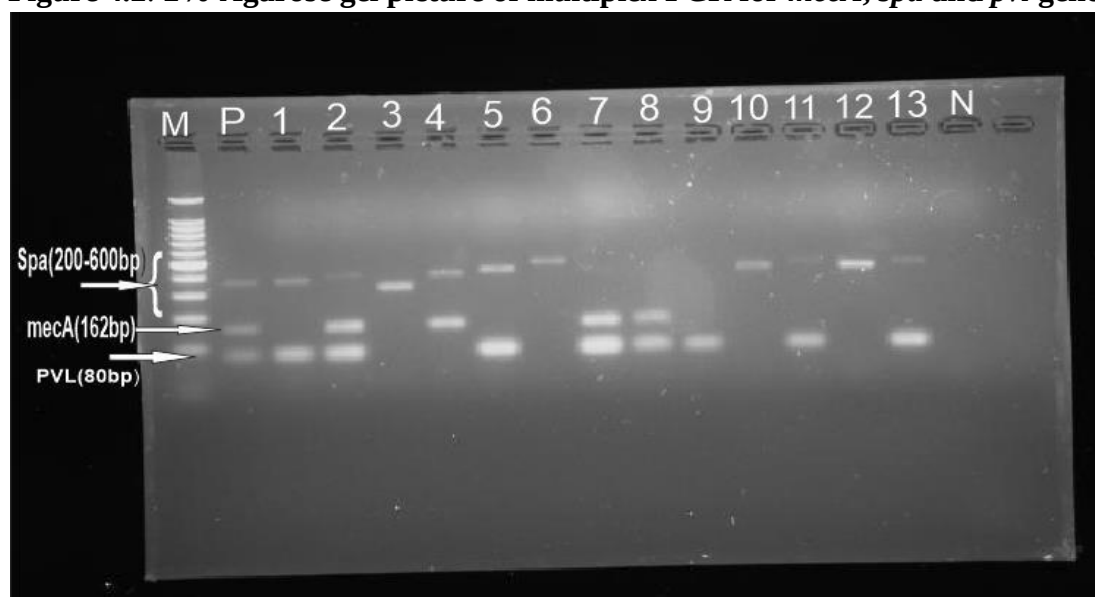


## 4.7 Molecular Characteristics of *Staphylococcus aureus* Isolates

### 4.7.1 Molecular Detection of *mecA*, *spa* and *pvl* genes

The *mecA* gene was detected in 4 (31%) of the 13 of the isolates. Eight (62%) of the 13 isolates were PVL-positive. One of the isolates showed all three genes. Figure 4.2 shows the PCR products on a 2% agarose gel.

**Figure 4.2: 2% Agarose gel picture of multiplex PCR for *mecA*, *spa* and *pvl* genes**



**Lane 1: M**-100-bp ladder; **Lane 2: P**- positive control Lane 3-15: **1-13**- *S. aureus* isolates recovered: **Lane 13: N**-negative control

### 4.7.2 *Spa*/Sequence type and Toxin Gene Distribution among *Staphylococcus aureus* Isolates

Four *spa* types were identified (t355; n=4, t002; n=1, t586 n=1, t2132 n=1). The prevalent *spa* type was t355 (30%). Five sequence types were also identified (ST152; n= 6, ST5; n= 3, ST30; n=1, ST3249; n=2, ST3777; n=1). The prevalent *S. aureus* clone was PVL-positive t355 (ST152). A summary of the *spa*/sequence types at the various hospitals and their *mecA* and *pvl* positivity can be found in Table 4.7.

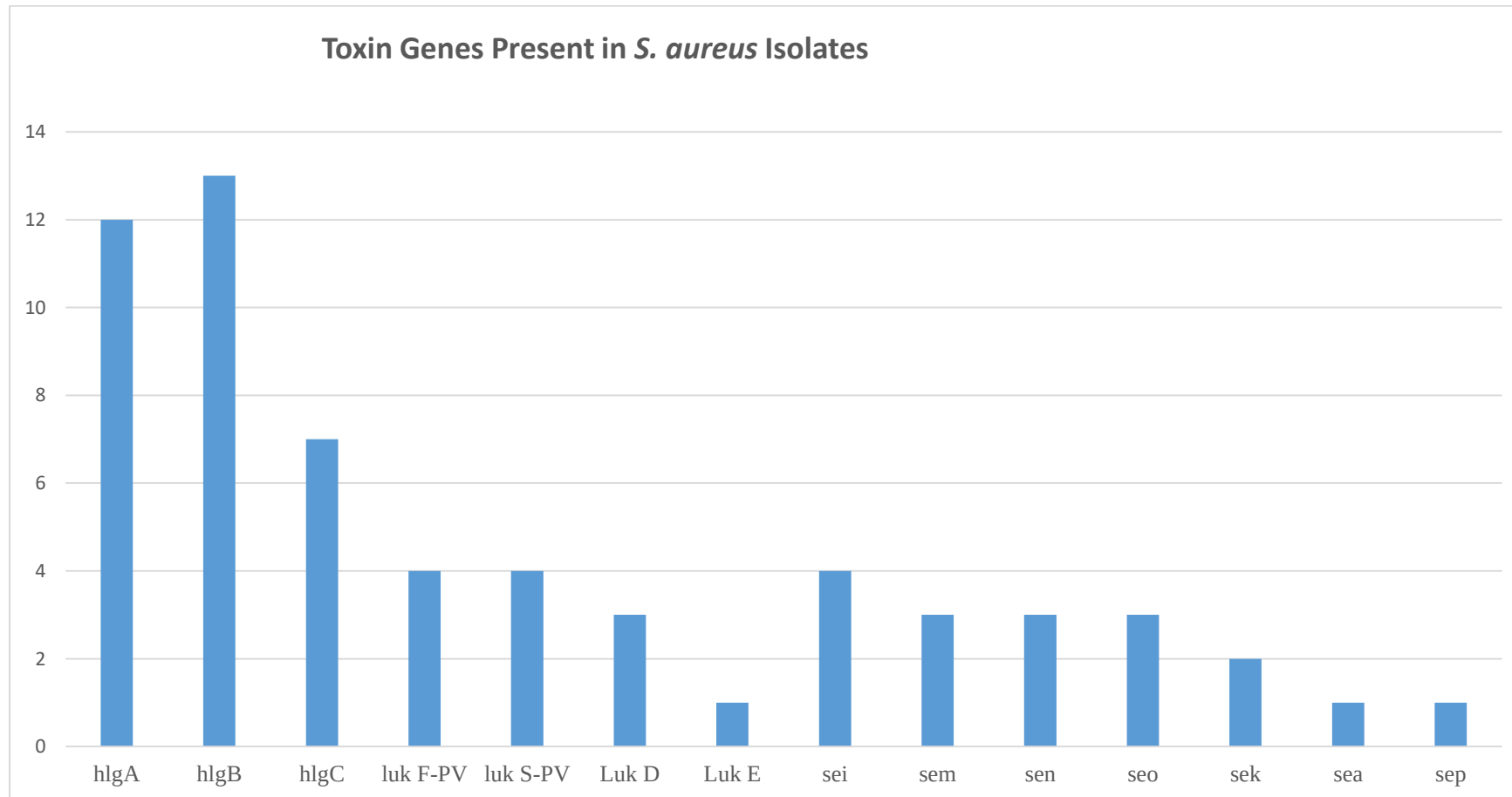
**Table 4.7: Distribution of *Spa*/Sequence type and PVL toxin among Hospitals**

| <i>spa</i> / Sequence type | 37-Military Hospital | Korle-Bu Teaching Hospital | MRSA/MSSA | PVL-positive |
|----------------------------|----------------------|----------------------------|-----------|--------------|
| t355 (ST152)               | 3                    | 0                          | MRSA      | 3/3          |
| t586 (ST5)                 | 1                    | 0                          | MRSA      | 0/1          |
| t355 (ST152)               | 0                    | 1                          | MSSA      | 0/1          |
| t002 (ST5)                 | 0                    | 1                          | MSSA      | 0/1          |
| t2132 (ST30)               | 1                    | 0                          | MSSA      | 0/1          |
| ST152                      | 0                    | 2                          | MSSA      | 2/2          |
| ST5                        | 1                    | 0                          | MSSA      | 1/1          |
| ST3249                     | 1                    | 1                          | MSSA      | 2/2          |
| ST3777                     | 1                    | 0                          | MSSA      | 0/1          |

#### 4.7.3 Virulence Factors for *S. aureus* isolates

Assembled genomes of the isolates were uploaded to the virulenceFinder 2.0 on the Center for Genomic Epidemiology webpage to identify toxin genes present in the isolates. The most prevalent toxin present was *hlgB* (100%), followed by *hlgA* (92.3%) and *hlgC* (53.8%). Four (30.7%) of the 13 isolates also possessed *lukF-PV* and *lukS-PV* which are the two genes encoding PVL. There were other enterotoxin genes present with *sei* (30.7%) being the most prevalent. Figure 4.3 gives a summary of the toxins present.

**Figure 4.3: Toxin Genes Present in *Staphylococcus aureus* Isolates**



#### 4.7.4 Toxin Genes Present in MRSA and MSSA Isolates

All toxins present in MRSA and MSSA isolates were also identified. Table 4.8 summarizes the toxins present in the MRSA and MSSA strains. All MRSA isolates carried the *hlgA* and *hlgB* toxin genes. Two the isolates carried *lukF-PV* and *lukS-PV*. One isolate carried nine different toxin genes.

**Table 4.8: Toxin Genes Present in MRSA and MSSA Isolates**

| MRSA        | Toxin Genes Present                                                                                                     | <i>spa</i> /sequence Type |
|-------------|-------------------------------------------------------------------------------------------------------------------------|---------------------------|
| Isolate 1   | <i>hlgA</i> + <i>hlgB</i> + <i>lukS-PV</i> + <i>lukF-PV</i>                                                             | t355 (ST152)              |
| Isolate 2   | <i>hlgA</i> + <i>hlgB</i> + <i>hlgC</i> + <i>lukD</i> + <i>sei</i> + <i>sem</i> + <i>sen</i> + <i>seo</i> + <i>sep</i>  | t586 (ST5)                |
| Isolate 3   | <i>hlgA</i> + <i>hlgB</i> + <i>lukS-PV</i> + <i>lukF-PV</i>                                                             | t355 (ST152)              |
| Isolate 4   | <i>hlgA</i> + <i>hlgB</i>                                                                                               | t355 (ST152)              |
| <b>MSSA</b> |                                                                                                                         |                           |
| Isolate 1   | <i>hlgA</i> + <i>hlgB</i> + <i>hlgC</i> + <i>LukE</i> + <i>LukD</i> + <i>sei</i> + <i>sem</i> + <i>sen</i> + <i>seo</i> | ST5                       |
| Isolate 2   | <i>hlgA</i> + <i>hlgB</i> + <i>sea</i> + <i>sek</i>                                                                     | ST3777                    |
| Isolate 3   | <i>hlgA</i> + <i>hlgB</i>                                                                                               | ST3249                    |
| Isolate 4   | <i>hlgA</i> + <i>hlgB</i> + <i>hlgC</i> + <i>sei</i> + <i>sek</i>                                                       | t2132 (ST30)              |
| Isolate 5   | <i>hlgA</i> + <i>hlgB</i> + <i>lukS-PV</i> + <i>lukF-PV</i>                                                             | ST152                     |
| Isolate 6   | <i>hlgA</i> + <i>hlgB</i>                                                                                               | t355 (ST152)              |
| Isolate 7   | <i>hlgB</i>                                                                                                             | ST3249                    |
| Isolate 8   | <i>hlgA</i> + <i>hlgB</i> + <i>lukS-PV</i> + <i>lukF-PV</i>                                                             | ST152                     |
| Isolate 9   | <i>hlgA</i> + <i>hlgB</i> + <i>hlgC</i> + <i>LukD</i> + <i>sei</i> + <i>sem</i> + <i>sen</i> + <i>seo</i>               | t002 (ST5)                |

#### 4.7.5 Antimicrobial Resistance patterns of MRSA and MSSA Isolates

Table 4.9 summarizes the resistance patterns shown by MRSA and MSSA isolates and the *spa*/sequence types they belong to. One MRSA isolate belonging to t586 (ST5) showed

resistance to four different antibiotics. On the other hand, 1 MSSA isolate belonging to ST3249 showed resistance to five different antibiotics while 1 isolate belonging to ST3777 showed resistance to three different antibiotics.

**Table 4.9: Antibiotype and *spa*/sequence types of MRSA and MSSA**

| Antibiotype           | Molecular Characteristics     |
|-----------------------|-------------------------------|
| Resistance Pattern    | <i>spa</i> /Sequence types    |
| <b>MRSA</b>           |                               |
| Fox+ P+ TE            | t355-ST152 (n=1)              |
| Fox+ P + TE+ LZD+ NOR | t586-ST5 (n=1)                |
| Fox+ P+ DA+ LZD+ERY   | t355-ST152 (n=1)              |
| Fox+ P+ ERY           | t355-ST152 (n=1)              |
| <b>MSSA</b>           |                               |
| P                     | ST3249 (n=1); ST152 (n=1)     |
| P+LZD                 | ST5 (n=1); t2132-ST30 (n=1)   |
| P+TE+ DA              | ST3777 (n=1)                  |
| P+TE+ LZD+RD+SXT      | ST3249 (n=1)                  |
| P+TE+ LZD             | ST152 (n=1); t355-ST152 (n=1) |
| P+ NOR                | t002-ST5 (n=1)                |

**Abbreviations:** Fox- Cefoxitin, P-Penicillin, TE-Tetracycline, LZD-Linezolid, NOR-Norfloxacin, DA-Clindamycin, ERY-Erythromycin, RD-Rifampicin, SXT-Co-trimoxazole

## CHAPTER FIVE

### 5.0 DISCUSSION

#### 5.1 Proportions of SSI Positive for *S. aureus*

Surgical site infections contribute to about 14-33% of all hospital acquired infection and is associated with substantial burden. There are a number of organisms that are part of the native flora known to cause SSI such as *Escherichia coli* and *Klebsiella pneumoniae*. However, the organism frequently isolated is *Staphylococcus aureus* and some studies have reported proportions between 18% and 39.7% (Dhar et al., 2014; Kahsay et al., 2014; Mpogoro et al., 2014; Takesue et al., 2017). In this study, the proportion of SSI positive for *S. aureus* (13/110; 11.8%) was significantly lower compared with a previous study conducted by Feglo & Afriyie-Asante (2014) at a private hospital in Obuasi where a proportion of 70/129 was reported (11.8% vs 54.2%,  $p < 0.001$ ). The variation observed in this study and Feglo & Afriyie-Asante (2014) study may be related to the fact that the hospital environment where the study was conducted was highly contaminated with organisms causing the infection. In addition, the CDC criteria which was used, was not followed by Feglo & Afriyie-Asante (2014) which could also account for the difference. Many other studies have also reported percentage proportions between 44-54% in Sudan, Nigeria and India (Ahmed, 2012; Isibor & Oseni, 2008; Kownhar et al., 2008). The disparities between the proportions of this study and the other studies could be explained by the fact that these studies either collected samples before antibiotic was administered (that had Gram-negative bacteria dominating), did not follow the CDC criteria or had bigger sample size compared to this study. The proportion reported in this study however, agrees with findings of another study by Malik et al. (2013) in a tertiary care hospital in Pakistan (11.8% vs 11%,  $p = 0.86$ ). Although the study by Dessie et al. (2016) found a proportion of 19/107 in Ethiopia, it is comparable to this study (17.7% vs 11.8%,  $p = 0.22$ ).

## 5.2 Antimicrobial Susceptibility Patterns of *S. aureus* Isolates

This study also sought to determine the antimicrobial resistance patterns of the isolates recovered, to guide treatment for patients with infections. Many documented studies have shown that the degree of resistance to commonly used antibiotics varies from country to country. However, it has been established that almost 90% of *S. aureus* strains are resistant to penicillin now (Sakoulas & Moellering, 2008). In Africa for instance, *S. aureus* strains have been identified to have a high level of resistance to penicillin (Schaumburg et al., 2014). However, in India, low sensitivity to penicillin, has been reported (Bhave et al., 2016; Kownhar et al., 2008). In this study, the isolates showed complete resistance to penicillin and this finding was in line with previous studies in Ghana and other African countries where this antibiotic has been tested (Akinkunmi et al., 2014; Feglo & Afriyie-Asante, 2014). In Ghana, penicillin was used to treat bacterial infections in the past, however, this drug is no more in use because of the development of resistance. This reflects the regular use of this antibiotic attributed to the fact that antibiotics are easily purchased with or without prescription from pharmacies. Flucloxacillin and clindamycin are now recommended for treating infections caused by *Staphylococcus spp*, as such, this finding has no bearing on clinical practice. However, our findings highlight the danger of inappropriate therapy for the treatment of infections. On the other hand, a high sensitivity rate was recorded for clindamycin which is consistent with what has been found in India (Bajaj et al., 2015; Naik & Deshpande, 2011; Ranjan et al., 2013) and Ethiopia (Ahmed et al., 2014). This indicates that clindamycin is still useful for staphylococcal treatment in Ghana. However, 2 (15.38%) of the isolates showed resistance to clindamycin which can be accounted for by its low frequent use before and after operation. Nonetheless, what this suggests is that, a high resistance can easily be developed and prudent use of this antibiotic is essential to prevent this.

It has been recognized that most bacteria develop resistance to relatively cheap and commonly used antibiotics. *S. aureus* strains in Africa have been known to have high rates of resistance to erythromycin, tetracycline and co-trimoxazole, owing to frequent prescription of these drugs in this setting (Schaumburg et al., 2014). Tetracycline resistance was not far from expected in this study. On the contrary, sensitivity to erythromycin and co-trimoxazole was high and recorded in 92.31% (12/13) of the isolates. Comparing the findings of this study to other studies in Ghana, it is similar to what has been found among nasal carriers of *S. aureus* (Egyir et al., 2013) and in contrast with the previous study on SSI (Feglo, & Afriyie-Asante, 2014). It would be hard to say what is accounting for the differences because nasal colonization with wound infection was not considered in this study. However, it may be attributed to the presence of organisms contaminating the hospital environment in the wounds of patients as in Feglo & Afriyie-Asante (2014) study. These organisms because they are in the hospital environment may have been frequently exposed to these antibiotics, thus, developed resistance (Feglo, & Afriyie-Asante, 2014). The study also found that the organisms in the wound and the hospital environment had similar antimicrobial susceptibility patterns further supporting this reasoning.

It is not surprising that high sensitivity was recorded for rifampicin 92.31% (12/13) and norfloxacin 76.92% (10/13). These antibiotics were not reported as part of the frequently used antibiotics among surgical patients in this study. Additionally, they have not been reported as part of the commonly used or prescribed antibiotics in KBTH (Labi et al., 2018). In other parts of the world, this antibiotic is prescribed for treating MRSA infections. Although in Ghana this is not the case, there are still clinical implications if rifampicin resistance occurs at a high rate. Most importantly, it can have a negative impact on chemotherapy for tuberculosis infections. These strains could easily transfer their acquired resistance to *Mycobacterium tuberculosis*, the organism responsible for tuberculosis and because this antibiotic is a primary



drug for the management of tuberculosis infections (CDC, 2016), it may pose therapeutic challenges.

The high rate of susceptibility to gentamicin in this study is in contrast with what was observed in the earlier study by Feglo & Afriye-Asante (2014) where 84% resistance was recorded as well as in other documented studies in Ethiopia and Nigeria. This difference could be explained by the fact that there has been a 26.6% reduction in the use of this antibiotic (Labi et al., 2018). As at 2017, this drug was not among the top five antibiotics frequently used at KBTH (Labi et al., 2018). Although in the year 2000 it was the fourth on the list (Newman, 2009). Yet, it is similar to what has been observed among patients with nasal colonisation of *S. aureus* (Egyir et al., 2013).

Vancomycin is used as the last line for treatment of MRSA infections (Gu et al., 2015), although reduced susceptibility to vancomycin has been reported in a few cases (Gardete & Tomasz, 2014; Kheder et al., 2012). In this study, all MRSA isolates showed complete susceptibility to Vancomycin which is in agreement with what has been reported worldwide in India, China and some African countries (Ahmed, 2012; Bhave et al., 2016; Gu et al., 2015; Kownhar et al., 2008; Naik & Deshpande, 2011; Ranjan et al., 2013; Seni et al., 2013). This finding mirrors the unavailability of this drug and its infrequent use in Ghana (Labi et al., 2018). More than half of the isolates 53.8% (7/13) were also resistant to linezolid, a drug used in for the treatment of MRSA infections (Gu et al., 2015). Although linezolid is not used in Ghana, the current findings indicate that linezolid may not be suitable for use. However, vancomycin as a last line drug for treatment MRSA infections remains an excellent choice in Ghana.

### 5.3 MRSA and MSSA among *S. aureus* Isolates

In Africa, MRSA has been reported to be <50% among *S. aureus* isolates (Schaumburg et al., 2014). Earlier studies in Ghana among nasal carriers of *S. aureus*, buruli and non-buruli ulcer patients and blood cultures have reported MRSA proportions of 1/56, 9/308 and 31/101 respectively (Dekker et al., 2016; Egyir et al., 2013; Kpeli et al., 2016) which supports the aforementioned fact. Amongst the 13 *S. aureus* recovered in this study, 4 isolates representing 30.76% were MRSA positive by both phenotypic screening and genotypic identification of the *mecA* gene. This reported proportion for MRSA further backs the <50% suggestion. Similar findings have been reported in Iran by Adwan et al., (2016) (30.76% vs 30%, p=0.89) among patients with SSI. The results also compares well with 37.5% (24/64) reported by Seni et al. (2013) (30.76% vs 37.50%, p=0.63) and 43.9% by Gu et al. (2015) (30.76% vs 43.9%, p=0.35) in Shanghai. Yet, it is much lower than 85.5% (47/55) (30.76% vs 85.5%, p<0.01) reported by Ahmed (2012) and in contrast to Mundhada & Tenpe (2015) study where no MRSA was recorded in 14 isolated *S. aureus*.

In comparing resistance to different antibiotics in MRSA and MSSA isolates, MRSA isolates showed resistance to at least 3 different antibiotics and majority of MSSA isolates to 1 antibiotic. The identification of a number of Multidrug resistant (MDR) isolates is not surprising. All over the world, MRSA has been known to be resistant to a range of antibiotics particularly penicillin and  $\beta$ -lactams (Kahsay et al., 2014). Contributing to this may be the fact that in Ghana and many African countries antibiotics can be obtained without prescriptions from pharmacies, as such, it is common practice for people to self-medicate. In addition to this, most patients do not complete their dosage and this leads to antibiotic selection. Thus, the finding that multidrug resistance is associated more with MRSA than MSSA is consistent with previous studies in Ghana, Uganda and Nigeria (Egyir et al., 2013; Seni et al., 2013; Shittu et al., 2011). This is a cause for concern because these MDR isolates could be

transferred to other organisms or non-carriers which could lead to subsequent dissemination (CDC, 2013).

#### **5.4 Molecular Epidemiology and Genetic Diversity of *S. aureus* Isolates**

The *S. aureus* isolates in this study were characterized using genotypic methods. To the best of our knowledge, this is the first study using sequencing to gain insight into the characteristics of *S. aureus* recovered from SSI in Ghana. Most SSI studies do not go beyond susceptibility testing and *mecA* detection. Thus, diversity of *S. aureus* among patients with surgical site infections in Ghana is not known. The few documented studies on *S. aureus* diversity have concentrated on samples from blood, skin and soft tissue infections and nasal carriage. This study therefore provides some baseline data on the diversity in of *S. aureus* in SSIs.

The results of the study show that the isolates carried the *spa* gene. Sequencing and subsequent *spa* typing showed that the isolates belonged to 4 *spa* types: t355, t002, t586 and t2132 while some were unknown. The *spa* type t355 was predominant and is known to be circulating in Ghana. Earlier studies identified this particular *spa* type in blood samples, skin and soft tissue infections (Dekker et al., 2016; Egyir et al., 2014), among nasal carriers of *S. aureus* (Egyir et al., 2013; Eibach et al., 2017), in burn wounds (Amissah et al., 2017) and both buruli ulcer and non-buruli ulcer patients (Amissah et al., 2015; Kpeli et al., 2016). This study thus supports the assertion that this is a well-established *spa* type in Ghana and in several African continents (Egyir et al., 2014; Schaumburg et al., 2014). The findings of this study are also consistent with results of a similar study by Seni et al. (2013) in Uganda.

*Spa* type t002 identified in this study is widely distributed and predominant in Europe Asia, USA, Canada and Brazil (Asadollahi et al., 2018; Grundmann et al., 2010). It has also been reported as the predominant type in bacteraemia caused by *S. aureus* in the United Kingdom (Satta et al., 2013). In Ghana, it has only been reported in patients with buruli ulcer (Amissah et al., 2015). In contrast, the *spa* type t586 was not identified in any of the literature reviewed

in Ghana. However, it has been reported in Germany from ventilator-associated pneumonia (Strommenger et al., 2006), in the USA from patients with bacteraemia (Park et al., 2017) and in Peru from collected blood samples (García et al., 2012). From literature and the database of Ridom *Spa* Server ([www.spaserver.ridom.de](http://www.spaserver.ridom.de)), it seems like this type has not been identified in Africa. However, it has been identified in other countries like Czech Republic, Iran, Spain, Croatia, Norway and the Netherlands. This study may very well be the first to identify it in Africa. According to the strain records of the Ridom *Spa* Server ([www.spaserver.ridom.de](http://www.spaserver.ridom.de)), t2132 was detected in Norway in 2007 and the Netherlands in 2013 and associated with MSSA as was consistent with our study. However, none of the above-mentioned studies from Ghana and Africa have isolated this *spa* type. This, therefore may be considered a new *spa* type in our region.

This study also identified five sequence types among MSSA and MRSA isolates. ST152 a typical African clone in Central and West Africa (Schaumburg et al., 2014) was predominant. Ruimy et al. (2008) also reported that this is a predominant type in Mali. There have also been previous reports of ST152 clone in clinical specimens from Nigeria, Gabon and Ghana (Ateba Ngoa et al., 2012; Dekker et al., 2016; Donkor et al., 2018; Egyir et al., 2014; Eibach et al., 2017; Kpeli et al., 2016; Okon et al., 2009). There appears to be a sustained association between *spa* types and sequence types regardless of the continent it is found on (Asadollahi et al., 2018; O'Hara et al., 2016). ST152 is most often associated with *spa* type t355 whether MSSA or MRSA and is widely distributed in African countries including Ghana and in central Europe (Breurec et al., 2011; Dekker et al., 2016; Donkor et al., 2018; Egyir et al., 2014; Ruimy et al., 2008).

Another sequence type found in this study ST5 has been reported among different clinical isolates in Ghana though not predominant (Donkor et al., 2018; Egyir et al., 2014). ST5-MRSA and ST5-MSSA are also prevalent in Central and West Africa (Schaumburg et al.,

2014) and have been reported in Gabon among nasal carriers (Ateba Ngoa et al., 2012). These clones are highly prevalent in the same geographic area and it has been suggested that ST5-MRSA may have evolved from ST5-MSSA during acquisition of the *SCCmec* element (Schaumburg et al., 2014). The clone t586-MRSA (ST5) which has been reported in healthy pre-school children in Belgium (Blumental et al., 2013) was also identified in this study.

There were two other sequence types identified in this study: ST30 and ST3249. ST30 is the common sequence type in Australia and has been described in some African countries. ST30-MSSA has been identified in South Africa, USA and Germany (Goering et al., 2008) and is a well disseminated clone. The clone t2132-MSSA (ST30) found in this study, was not identified in literature, probably because t2132 has only been found in Norway and the Netherlands and no studies have been published on it. ST3249 is a new sequence type previously identified in a burn unit in Ghana (Amissah et al., 2017). There are no present reports of ST3777 in Ghana. Literature search encountered did not produce any report(s) anywhere else.

Eight (61.5%) PVL-positive strains were encountered in this study, 3 MRSA and 5 MSSA. The presence of high proportions of PVL-positives is not surprising because Africa has been described as an endemic region for PVL especially central and West Africa (Breurec et al., 2011; Rasigade et al., 2014; Schaumburg et al., 2014). This is in stark contrast with the Europe, USA and Asia where PVL is rare especially in MSSA isolates (Egyir et al., 2014; Rasigade et al., 2014). The results of ST152-MSSA harbouring the *pvl* gene is thus consistent with reports in African countries like Mali and Nigeria (Ruimy et al., 2008; Shittu et al., 2011). In Ghana, PVL-positive t355(ST152) MSSA, a clone most often isolated from skin and soft tissue infections has been reported in earlier studies from clinical samples (Egyir et al., 2014). However, the study did not find any PVL-positive ST152-MRSA in the over 300 *S. aureus* isolates recovered. Thus, finding this clone, especially in this study is remarkable. This is a

pandemic clone that has been associated with PVL-positive CA-MRSA in Central Europe, the Balkan, Switzerland (Monecke et al., 2007; Ruimy et al., 2008) and found in healthy people in the community with no exposure to the healthcare setting (Müller-Premru et al., 2005). Since PVL-MRSA is associated with significant infections in patients without any risk factors (Linde et al., 2005), this finding is of concern. It has been proposed that the constant association of ST152 with PVL raises the possibility that it is the original clone which acquired *pvl* and was disseminated to the *S. aureus* population. The PVL-positive clone t355 (ST152) MRSA has also been reported from an immigrant child from Macedonia (Monecke et al., 2007).

It has been said that ST5 rarely carries the *pvl* gene, but up to 33% of strains belonging to this type carry it (Breurec et al., 2011). One of the 2 ST5-MSSA in this study carried the *pvl* gene which was consistent with reports by Egyir et al., (2014). ST5-MRSA harbouring the *pvl* gene has also been reported in other African countries (Breurec et al., 2011), however, this was not the case in our study. On the other hand, ST30 is said to have high proportions of *pvl* (Schaumburg et al., 2014). Yet, ST30-MSSA in this study did not carry the *pvl* gene. This finding is also in stark contrast to what has been reported in Ghana (Egyir et al., 2014). A possible explanation cannot be provided for this contrast but may only imply that some ST30-MSSA strains in SSI may not carry the toxin.

The possession of a host of virulence factor is a major way *S. aureus* colonises its host and causes disease. The enterotoxins (SEA, SEI, SEM, SEN, SEO, SEK, SEP), Leukocidin (E and D) and  $\gamma$  toxins (HlgA, HlgB and HlgC) identified in this study allow for invasion and damage of host tissues (Reygaert, 2013) and are associated with certain diseases. PVL, Leukocidin (E and D) and  $\gamma$  toxins (HlgA, HlgB and HlgC) have been suggested to contribute to enhanced virulence. This study has shown that the *S. aureus* isolates carry at least two virulence genes

which was consistent with a study in Shanghai (Gu et al., 2015). This is of concern especially among the resistant isolates because it may pose a big challenge to treatment of infections.

## CHAPTER SIX

### 6.0 CONCLUSION, RECOMMENDATION, LIMITATIONS

#### 6.1 Conclusion

The results obtained from this study have shown that the proportion of SSI positive for *S. aureus* at Korle-Bu Teaching Hospital and 37-military Hospital is 13/110 (11.8%). MRSA accounted for 30.77% (4/13) of the total *S. aureus* isolates. In addition, these *S. aureus* isolates are highly resistant to penicillin but susceptible to gentamicin and vancomycin which implies that gentamicin can be used for the treatment of *S. aureus* infections and vancomycin is viable as a last line drug for the treatment of *S. aureus* and MRSA infections. Additionally, *Staphylococcus aureus* isolates in the two hospitals are genetically diverse belonging mostly to t355 (ST152) and carry the *pvl* gene believed to be associated with CA-MRSA, severe infections and necrosis as well as other toxin genes like *hlgA*, *hglB*, *hlgC* and *sei*. t586 (ST5) and t2132 (ST30) PVL-negative strains and ST3777 identified have previously not been detected in Ghana. This study has provided insight into the molecular characteristics of *S. aureus* from SSIs in Ghana which have not been done in previous studies.

#### 6.2 Recommendations

1. Continuous surveillance and infection control practices should be established to monitor and control the spread of MRSA in the two hospitals.
2. Routine detection of MRSA should be done in the hospitals even if with just phenotypic testing and antimicrobial susceptibility performed to guide treatment of infections.
3. Studies covering more hospitals, a larger sample size and other organisms should be conducted to give a picture of what is going on with SSI and what strains are circulating in the hospitals.



4. Studies comparing nasal carriage of *S. aureus* and isolates recovered from surgical site infections should also be conducted to determine whether they are the same strains and if nasal decolonization is necessary before surgery.

### **6.3 Limitations**

1. There were difficulties in using the CDC guideline because the hospitals did not have standard guidelines for SSI. This resulted in many patients being missed, hence, there was a limited number to work with.

## REFERENCES

- About us – Brief History – Korle-Bu Teaching Hospital. (n.d.). Retrieved July 30, 2019, from <http://kbth.gov.gh/brief-history/>
- Adwan, G., Abu Hasan, N., Sabra, I., Sabra, D., Al-butmah, S., Odeh, S., ... & Badran, H. (2016). Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. *International Journal of Medical Research and Health Sciences*, 5(5), 75–82.
- Ahmed, E. F., Gad, G. F. M., Abdalla, A. M., Hasaneen, A. M., & Abdelwahab, S. F. (2014). Prevalence of Methicillin Resistant Staphylococcus aureus among Egyptian Patients after Surgical Interventions. *Surgical Infections*, 15(4), 404–411.
- Ahmed, M. (2012). Prevalence of nosocomial wound infection among postoperative patients and antibiotics patterns at teaching hospital in Sudan. *North American Journal of Medical Sciences*, 4(1), 29.
- Akinkunmi, E. O., Adesunkanmi, A.-R., & Lamikanra, A. (2014). Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. *African Health Sciences*, 14(4), 802–9.
- Allegranzi, B., Bagheri Nejad, S., Combescure, C., Graafmans, W., Attar, H., Donaldson, L., & Pittet, D. (2011). Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *Lancet (London, England)*, 377(9761), 228–41.
- Ameyaw, J. A. O. (2014). *Surgical Site Infection After Abdominal Surgery: Prevalence, causes and management at the surgical wards, Komfo Anokye Teaching Hospital, Ghana*. (Masters thesis). Kwame Nkrumah University of Science and Technology, Ghana, Accra.
- Amissah, N. A., Glasner, C., Ablordey, A., Tetteh, C. S., Kotey, N. K., Prah, I., ... Stienstra, Y. (2015). Genetic Diversity of Staphylococcus aureus in Buruli Ulcer. *PLoS Neglected Tropical Diseases*, 9(2), e0003421.
- Amissah, N. A., Van Dam, L., Ablordey, A., Ampomah, O. W., Prah, I., Tetteh, C. S., ... Stienstra, Y. (2017). Epidemiology of Staphylococcus aureus in a burn unit of a tertiary care center in Ghana. *PLoS ONE*, 12(7), e0181072.
- Anderson, D. J. (2011). Surgical Site Infections. *Infectious Disease Clinics of North America*, 25(1), 135–153.
- Anderson, D. J., Podgorny, K., Berríos-Torres, S. I., Bratzler, D. W., Dellinger, E. P., Greene, L., ... Kaye, K. S. (2014). Strategies to Prevent Surgical Site Infections in Acute Care Hospitals: 2014 Update. *Infection Control & Hospital Epidemiology*, 35(S2), S66–S88.
- Apanga, S., Adda, J., Issahaku, M., Amofa, J., & Ama, K. R. (2014). Post-Operative Surgical Site Infection in a Surgical Ward of a Tertiary Care Hospital in Northern Ghana. *International Journal of Research in Health Sciences*, 2(1), 207–12.
- Asadollahi, P., Farahani, N. N., Mirzaii, M., Khoramrooz, S. S., van Belkum, A., Asadollahi, K., ... Darban-Sarokhalil, D. (2018). Distribution of the most prevalent spa types among clinical isolates of methicillin-resistant and -susceptible Staphylococcus aureus around the

- world: A review. *Frontiers in Microbiology*, 9, 163.
- Ateba Ngoa, U., Schaumburg, F., Adegnika, A. A., Kösters, K., Möller, T., Fernandes, J. F., ... Lell, B. (2012). Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa. *Acta Tropica*, 124(1), 42–47.
- Awad, S. S. (2012). Adherence to Surgical Care Improvement Project Measures and PostOperative Surgical Site Infections. *Surgical Infections*, 13(4), 234–237.
- Bajaj, A., Kukanur, S., Kotigadde, S., & Meundi, M. (2015). Gram Positive Cocci Causing Surgical Site Infection: Identification and Antibiotic Susceptibility Pattern. *IOSR Journal of Dental and Medical Sciences*, 14(8), 62–67.
- Bastola, R., Parajuli, P., Neupane, A., & Paudel, A. (2017). Surgical Site infections: Distribution Studies of Sample, Outcome and Antimicrobial Susceptibility Testing. *Journal of Medical Microbiology & Diagnosis*, 06(01), 2161-703.
- Behjati, S., & Tarpey, P. S. (2013). What is next generation sequencing? *Archives of Disease in Childhood. Education and Practice Edition*, 98(6), 236.
- Bercion, R., Gaudeuille, A., Mapouka, P. A., Behounde, T., & Guetahoun, Y. (2007). [Surgical site infection survey in the orthopaedic surgery department of the “Hôpital communautaire de Bangui,” Central African Republic]. *Bulletin de La Societe de Pathologie Exotique* (1990), 100(3), 197–200.
- Bhave, P., Kartikeyan, S., Ramteerthakar, M., & Patil, N. (2016). Bacteriological study of surgical site infections in a tertiary care hospital at Miraj, Maharashtra state, India. *International Journal of Research in Medical Sciences*, 2630–2635.
- Blaiotta, G., Fusco, V., von Eiff, C., Villani, F., & Becker, K. (2006). Biotyping of enterotoxigenic *Staphylococcus aureus* by enterotoxin gene cluster (egc) polymorphism and spa typing analyses. *Applied and Environmental Microbiology*, 72(9), 6117–23.
- Blumental, S., Deplano, A., Jourdain, S., De Mendonça, R., Hallin, M., Nonhoff, C., ... Denis, O. (2013). Dynamic pattern and genotypic diversity of *Staphylococcus aureus* nasopharyngeal carriage in healthy pre-school children. *Journal of Antimicrobial Chemotherapy*, 68(7), 1517–1523.
- Bratu, S., Eramo, A., Kopeck, R., Coughlin, E., Ghitan, M., Yost, R., ... Quale, J. (2005). Community-associated methicillin-resistant *Staphylococcus aureus* in hospital nursery and maternity units. *Emerging Infectious Diseases*, 11(6), 808–13.
- Breurec, S., Fall, C., Pouillot, R., Boisier, P., Brisse, S., Diene-Sarr, F., ... Laurent, F. (2011). Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: High prevalence of Panton-Valentine leukocidin genes. *Clinical Microbiology and Infection*, 17(4), 633–639.
- Centers for Disease Control and Prevention (CDC). (2013). Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, US Department of Health and Human Services.
- Centers for Disease Control and Prevention (CDC). (2016). Treatment of TB Diseases. Atlanta, GA: CDC.

Retrieved October 2020 from  
[https://www.cdc.gov/tb/topic/treatment/tbdisease.htm#:~:text=Of%20the%20approved%20drugs%2C%20the,ethambutol%20\(EMB\)](https://www.cdc.gov/tb/topic/treatment/tbdisease.htm#:~:text=Of%20the%20approved%20drugs%2C%20the,ethambutol%20(EMB))

- Centers for Disease Control and Prevention (CDC). (2015). National Healthcare Safety Network (NHSN). Surgical Site Infection (SSI) event. Procedure-associated module SSI. Atlanta, GA: CDC. Retrieved March 13 2020
- Centers for Disease Control and Prevention (CDC). (2019). National Healthcare Safety Network (NHSN). Surgical Site Infection (SSI) event. Procedure-associated module SSI. Atlanta, GA: CDC. Retrieved March 13 2019 from [www.cdc.gov/nhsn/pdfs/psc-manual/9pscscsscurrent.pdf](http://www.cdc.gov/nhsn/pdfs/psc-manual/9pscscsscurrent.pdf).
- Chambers, H. F. (1997). Methicillin resistance in staphylococci: Molecular and biochemical basis and clinical implications. *Clinical Microbiology Reviews*, 10(4), 781-791.
- Chambers, H. F., & DeLeo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nature Reviews Microbiology*, 7(9), 629.
- Clyne, M., De Azavedo, J., Carlson, E., & Arbuthnott, J. (1988). Production of gamma-hemolysin and lack of production of alpha-hemolysin by Staphylococcus aureus strains associated with toxic shock syndrome. *Journal of Clinical Microbiology*, 26(3), 535-9.
- Cosgrove, S. E., Sakoulas, G., Perencevich, E. N., Schwaber, M. J., Karchmer, A. W., & Carmeli, Y. (2003). Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Bacteremia: A Meta-analysis. *Clinical Infectious Diseases*, 36(1), 53-59.
- Crossley, K. B., & Archer, G. L. (1997). *The staphylococci in human disease*. New York, NY: Churchill Livingstone, Inc.
- Crossley, K. B., Jefferson, K. K., Archer, G. L., & Fowler, V. G. (2009). *Staphylococci in Human Disease: (Second Edition)*. New Jersey, NJ: John Wiley & Sons, Inc.
- Cuny, C., Wieler, L., & Witte, W. (2015). Livestock-Associated MRSA: The Impact on Humans. *Antibiotics*, 4(4), 521-543.
- Dashti, A., Jadaon, M., Abdulsamad, A., & Dashti, M. (2009). Heat Treatment of Bacteria: A Simple Method of DNA Extraction for Molecular Techniques. *Kuwait Medical Journal*, 41(2), 117-122.
- Dauwalder, O., Thomas, D., Ferry, T., Debard, A.-L., Badiou, C., Vandenesch, F., ... Monneret, G. (2006). Comparative inflammatory properties of staphylococcal superantigenic enterotoxins SEA and SEG: implications for septic shock. *Journal of Leukocyte Biology*, 80(4), 753-758.
- David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews*, 23(3), 616-87.
- Dekker, D., Wolters, M., Mertens, E., Boahen, K. G., Krumkamp, R., Eibach, D., ... May, J. (2016). Antibiotic resistance and clonal diversity of invasive Staphylococcus aureus in the rural Ashanti Region, Ghana. *BMC Infectious Diseases*, 16(1), 720.

- DeLeo, F. R., Otto, M., Kreiswirth, B. N., & Chambers, H. F. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*. *The Lancet*, 375(9725), 1557–1568.
- Dessie, W., Mulugeta, G., Fentaw, S., Mihret, A., Hassen, M., & Abebe, E. (2016). Pattern of bacterial pathogens and their susceptibility isolated from surgical site infections at selected referral hospitals, Addis Ababa, Ethiopia. *International Journal of Microbiology*, 2016, 1–8.
- Deurenberg, R. H., & Stobberingh, E. E. (2008). The evolution of *Staphylococcus aureus*. *Infection, Genetics and Evolution*, 8(6), 747–763.
- Dhar, H., Al-Busaidi, I., Rathi, B., Nimre, E. A., Sachdeva, V., & Hamdi, I. (2014). A study of post-caesarean section wound infections in a regional referral hospital, oman. *Sultan Qaboos University Medical Journal*, 14(2), e211-7.
- Donkor, E. S., Dayie, N. T. K. D., & Tette, E. M. A. (2019). Methicillin-Resistant *Staphylococcus aureus* in Ghana: Past, Present, and Future. *Microbial Drug Resistance*, 25(5), 717–724.
- Donkor, E. S., Jamrozy, D., Mills, R. O., Dankwah, T., Amoo, P. K., Egyir, B., ... Bentley, S. D. (2018). A genomic infection control study for *Staphylococcus aureus* in two Ghanaian hospitals. *Infection and Drug Resistance*, 11, 1757–1765.
- Dufour, P., Gillet, Y., Bes, M., Lina, G., Vandenesch, F., Floret, D., ... Richet, H. (2002). Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Infections in France: Emergence of a Single Clone That Produces Panton-Valentine Leukocidin. *Clinical Infectious Diseases*, 35(7), 819–824.
- ECDC, E. (2009). The bacterial challenge: Time to react a Call to Narrow the Gap between Multidrug-Resistant Bacteria in the EU and Development of New Antibacterial Agents. Stockholm: ECDC & EMEA Joint Press Release.
- Egyir, B., Guardabassi, L., Esson, J., Nielsen, S. S., Newman, M. J., Addo, K. K., & Larsen, A. R. (2014). Insights into nasal carriage of *Staphylococcus aureus* in an urban and a rural community in Ghana. *PLoS ONE*, 9(4), e96119.
- Egyir, B., Guardabassi, L., Nielsen, S. S., Larsen, J., Addo, K. K., Newman, M. J., & Larsen, A. R. (2013). Prevalence of nasal carriage and diversity of *Staphylococcus aureus* among inpatients and hospital staff at Korle Bu Teaching Hospital, Ghana. *Journal of Global Antimicrobial Resistance*, 1(4), 189–193.
- Egyir, B., Guardabassi, L., Sørum, M., Nielsen, S. S., Kolekang, A., Frimpong, E., ... Larsen, A. R. (2014). Molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. *PLoS ONE*, 9(2), e89716.
- Eibach, D., Nagel, M., Hogan, B., Azuure, C., Krumkamp, R., Dekker, D., ... May, J. (2017). Nasal Carriage of *Staphylococcus aureus* among Children in the Ashanti Region of Ghana. *PLOS ONE*, 12(1), e0170320.
- Eriksen, H. ., Chugulu, S., Kondo, S., & Lingaas, E. (2003). Surgical-site infections at Kilimanjaro Christian Medical Center. *Journal of Hospital Infection*, 55(1), 14–20.
- European Centre for Disease Prevention and Control. (2015). *European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Annual*

*Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2015. Reproduction.*

- Falagas, M. E., Karageorgopoulos, D. E., Leptidis, J., & Korbila, I. P. (2013). MRSA in Africa: Filling the Global Map of Antimicrobial Resistance. *PLoS ONE*, 8(7), e68024.
- Fan, Y., Wei, Z., Wang, W., Tan, L., Jiang, H., Tian, L., ... Nie, S. (2014). The incidence and distribution of surgical site infection in mainland China: A meta-analysis of 84 prospective observational studies. *Scientific Reports*, 4(1), 6783.
- Farrington, M. (2012). Antibacterial drugs. In *Clinical Pharmacology: Eleventh Edition* (pp. 173–190). Churchill Livingstone.
- Feglo, P., & Afriyie-Asante, A. (2014). Environmental impact on postoperative wound infections in a privately owned hospital in Ghana. *African Journal of Microbiology Research*, 8(15), 1620–1626.
- Fehr, J., Hatz, C., Soka, I., Kibatala, P., Urassa, H., Smith, T., ... Widmer, A. (2006). Risk Factors for Surgical Site Infection in a Tanzanian District Hospital: A Challenge for the Traditional National Nosocomial Infections Surveillance System Index. *Infection Control & Hospital Epidemiology*, 27(12), 1401–1404.
- Forbes, B. A., Sahm, D. F., Weissfeld, A. S., & Bailey, W. R. (William R. (2007). *Bailey & Scott's diagnostic microbiology*. USA: Elsevier Mosby.
- Foster, T. (1996). Staphylococcus. In *Medical Microbiology*. 4<sup>th</sup> edition. University of Texas Medical Branch at Galveston.
- Foster, T. J. (2004). The Staphylococcus aureus “superbug.” *Journal of Clinical Investigation*, 114(12), 1693–1696.
- García, C., Rijnders, M. I. A., Bruggeman, C., Samalvides, F., Stobberingh, E. E., & Jacobs, J. (2012). Antimicrobial resistance and molecular typing of Staphylococcus aureus bloodstream isolates from hospitals in Peru. *Journal of Infection*, 65(5), 406–411.
- Gardete, S., & Tomasz, A. (2014). Mechanisms of vancomycin resistance in Staphylococcus aureus. *Journal of Clinical Investigation*, 124(7), 2836–2840.
- Garnier, F., Tristan, A., François, B., Etienne, J., Delage-Corre, M., Martin, C., ... Ploy, M.-C. (2006). Pneumonia and new methicillin-resistant Staphylococcus aureus clone. *Emerging Infectious Diseases*, 12(3), 498–500.
- Goering, R. V., Shawar, R. M., Scangarella, N. E., O'Hara, F. P., Amrine-Madsen, H., West, J. M., ... Twynholm, M. E. (2008). Molecular epidemiology of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolates from global clinical trials. *Journal of Clinical Microbiology*, 46(9), 2842–7.
- Gordon, N. C., Price, J. R., Cole, K., Everitt, R., Morgan, M., Finney, J., ... Golubchik, T. (2014). Prediction of Staphylococcus aureus antimicrobial resistance by whole-genome sequencing. *Journal of Clinical Microbiology*, 52(4), 1182–91.
- Gorwitz, R. J. (2008). Understanding the Success of Methicillin-Resistant Staphylococcus aureus Strains Causing Epidemic Disease in the Community. *The Journal of Infectious Diseases*, 197(2), 179–182.

- Grimble, S. A. ., Magee, T. ., & Galland, R. . (2001). Methicillin Resistant *Staphylococcus aureus* in Patients Undergoing Major Amputation. *European Journal of Vascular and Endovascular Surgery*, 22(3), 215–218.
- Grundmann, H., Aanensen, D. M., Van Den Wijngaard, C. C., Spratt, B. G., Harmsen, D., Friedrich, A. W., ... Vogel, U. (2010). Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: A molecular-epidemiological analysis. *PLoS Medicine*, 7(1), e1000215.
- Gu, F.-F., Han, L.-Z., Chen, X., Wang, Y.-C., Shen, H., Wang, J.-Q., ... Ni, Y.-X. (2015). Molecular Characterization of *Staphylococcus aureus* from Surgical Site Infections in Orthopedic Patients in an Orthopedic Trauma Clinical Medical Center in Shanghai. *Surgical Infections*, 16(1), 97–104.
- Harris, S. R., Cartwright, E. J., Török, M. E., Holden, M. T., Brown, N. M., Ogilvy-Stuart, A. L., ... Peacock, S. J. (2013). Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. *The Lancet Infectious Diseases*, 13(2), 130–136.
- Hetem, D. J., Rooijackers, S. H. M., & Ekkelenkamp, M. B. (2016). *Staphylococci and Micrococci*. In *Infectious Diseases* (p. 1509–1522.e2).
- Hodzic, J., Gurbeta, L., Omanovic-Miklicanin, E., & Badnjevic, A. (2017). Overview of Nextgeneration Sequencing Platforms Used in Published Draft Plant Genomes in Light of Genotypization of Immortelle Plant (*Helichrysium Arenarium*). *Medical Archives*, 71(4), 288.
- Holtfreter, S., Grumann, D., Schmudde, M., Nguyen, H. T. T., Eichler, P., Strommenger, B., ... Bröker, B. M. (2007). Clonal distribution of superantigen genes in clinical *Staphylococcus aureus* isolates. *Journal of Clinical Microbiology*, 45(8), 2669–2680.
- Hsu, L.-Y., Tristan, A., Koh, T.-H., Bes, M., Etienne, J., Kurup, A., ... Tan, B.-H. (2005). Community associated methicillin-resistant *Staphylococcus aureus*, Singapore. *Emerging Infectious Diseases*, 11(2), 341–2.
- Humphreys, M. (2018). The influenza of 1918: Evolutionary perspectives in a historical context. *Evolution, Medicine, and Public Health*, 2018(1), 219–229.
- Illumina, I. (2015). An introduction to next-generation sequencing technology.
- Isibor, J., & Oseni, A. (2008). Incidence of aerobic bacteria and *Candida albicans* in postoperative wound infections. *African Journal of Microbiology Research*, 2(11), 288–291.
- Iyamba, J. M. L., Wambale, J. M., Lukukula, C. M., & Takaisi-Kikuni, N. za B. (2014). High prevalence of methicillin resistant staphylococci strains isolated from surgical site infections in Kinshasa. *Pan African Medical Journal*, 18, 322.
- Johnson, A. P. (2011). Methicillin-resistant *Staphylococcus aureus*: The European landscape. *Journal of Antimicrobial Chemotherapy*, 66(suppl\_4), iv43-iv48.
- Kahsay, A., Mihret, A., Abebe, T., & Andualem, T. (2014). Isolation and antimicrobial susceptibility pattern of *Staphylococcus aureus* in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region, Ethiopia. *Archives of Public Health*, 72(1).

- Karikari, A. B., Frimpong, E., & Owusu-Ofori, A. (2017). Methicillin-resistant *Staphylococcus aureus* among patients in a teaching hospital in Ghana. *International Journal of One Health*, 3, 46–49.
- Kesah, C., Redjeb, S. Ben, Odugbemi, T. O., Boye, C. S.-B., Dosso, M., Ndinya Achola, J. O., ... Borg, M. (2003). Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. *Clinical Microbiology and Infection*, 9(2), 153–156.
- Kheder, S. I., Ali, N. A., & Fathelrahman, A. I. (2012). Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistance *Staphylococcus* in a Sudanese Surgical Ward. *Pharmacology & Pharmacy*, 03(01), 103–108.
- Klevens, R. M., Edwards, J. R., Richards, C. L., Horan, T. C., Gaynes, R. P., Pollock, D. A., & Cardo, D. M. (2007). Estimating Health Care-Associated Infections and Deaths in U.S. Hospitals, 2002. *Public Health Reports*, 122(2), 160–166.
- Knobler, S. L., Lemon, S. M., Najafi, M., & Burroughs, T. (2003). Strategies to Contain the Development and Consequences of Resistance. In *The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment: Workshop Summary*. National Academies Press (US).
- Kong, Z., Zhao, P., Liu, H., Yu, X., Qin, Y., Su, Z., ... Chen, J. (2016). Whole-Genome Sequencing for the Investigation of a Hospital Outbreak of MRSA in China. *PLOS ONE*, 11(3), e0149844.
- Kotisso, B., & Aseffa, A. (1998). Surgical wound infection in a teaching hospital in Ethiopia. *East African Medical Journal*, 75(7), 402–5.
- Kownhar, H., Shankar, E. M., Vignesh, R., Sekar, R., Velu, V., & Rao, U. A. (2008). High isolation rate of *Staphylococcus aureus* from surgical site infections in an Indian hospital. *Journal of Antimicrobial Chemotherapy*, 61(3), 758–760.
- Kpeli, G., Darko Otchere, I., Lamelas, A., Buultjens, A. L., Bulach, D., Baines, S. L., ... Yeboah-Manu, D. (2016). Possible healthcare-associated transmission as a cause of secondary infection and population structure of *Staphylococcus aureus* isolates from two wound treatment centres in Ghana. *New Microbes and New Infections*, 13, 92–101.
- Kwong, J. C., McCallum, N., Sintchenko, V., & Howden, B. P. (2015). Whole genome sequencing in clinical and public health microbiology. *Pathology*, 47(3), 199–210.
- Labi, A.-K., Obeng-Nkrumah, N., Owusu, E., Bjerrum, S., Bediako-Bowan, A., Sunkwa-Mills, G., ... Newman, M. J. (2019). Multi-centre point-prevalence survey of hospital-acquired infections in Ghana. *Journal of Hospital Infection*, 101(1), 60–68.
- Labi, A. K., Obeng-Nkrumah, N., Nartey, E. T., Bjerrum, S., Adu-Aryee, N. A., Ofori-Adjei, Y. A., ... Newman, M. J. (2018). Antibiotic use in a tertiary healthcare facility in Ghana: A point prevalence survey. *Antimicrobial Resistance and Infection Control*, 7(1), 15.
- Larsen, A. R., Stegger, M., & Sørum, M. (2008). spa typing directly from a mecA, spa and pvl multiplex PCR assay-a cost-effective improvement for methicillin-resistant *Staphylococcus aureus* surveillance. *Clinical Microbiology and Infection*, 14(6), 611–614.
- Laryea, D. O., Amoako, Y. A., & Asamoah, J. (2014). Antibiotic Sensitivity and Clinical Outcome for Methicillin Resistant *Staphylococcus Aureus*. *Online Journal of Public Health Informatics*, 6(1).



- Levy, P.-Y., Ollivier, M., Drancourt, M., Raoult, D., & Argenson, J.-N. (2013). Relation between nasal carriage of *Staphylococcus aureus* and surgical site infection in orthopedic surgery: The role of nasal contamination. A systematic literature review and metaanalysis. *Orthopaedics & Traumatology: Surgery & Research*, 99(6), 645–651.
- Lilani, S. P., Jangale, N., Chowdhary, A., & Daver, G. B. (2005). Surgical site infection in clean and clean-contaminated cases. *Indian Journal of Medical Microbiology*, 23(4), 249–52.
- Linde, H., Wagenlehner, F., Strommenger, B., Drubel, I., Tanzer, J., Reischl, U., ... Lehn, N. (2005). Healthcare-associated outbreaks and community-acquired infections due to MRSA carrying the Panton-Valentine leucocidin gene in southeastern Germany. *European Journal of Clinical Microbiology and Infectious Diseases*, 24(6), 419–422.
- Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., ... Fridkin, S. K. (2014). Multistate Point-Prevalence Survey of Health Care–Associated Infections. *New England Journal of Medicine*, 370(13), 1198–1208.
- Magill, S. S., Hellinger, W., Cohen, J., Kay, R., Bailey, C., Boland, B., ... Fridkin, S. (2012). Prevalence of Healthcare-Associated Infections in Acute Care Hospitals in Jacksonville, Florida. *Infection Control & Hospital Epidemiology*, 33(03), 283–291.
- Malik, Z. I., Nawaz, T., Abdullah, M. T., Waqar, S. H., & Zahid, M. A. (2013). Surgical Site Infections in General Surgical Wards at a Tertiary Care Hospital. *Pakistan Journal of Medical Research* 52(4), 116.
- Mangram, A. J., Horan, T. C., Pearson, M. L., Silver, L. C., & Jarvis, W. R. (1999). Guideline for Prevention of Surgical Site Infection, 1999. *Infection Control & Hospital Epidemiology*, 20(4), 247–280.
- Marimuthu, K., Eisenring, M.-C., Harbarth, S., & Troillet, N. (2016). Epidemiology of *Staphylococcus aureus* Surgical Site Infections. *Surgical Infections*, 17(2), 229–235.
- Maza, L. D. la, Pezzlo, M., Shigei, J., & Tan, G. (2004). *Color atlas of medical bacteriology*. (1st ed.). Washington, D.C: American Society for Microbiology Press.
- Merkow, R. P., Ju, M. H., Chung, J. W., Hall, B. L., Cohen, M. E., Williams, M. V., ... Bilimoria, K. Y. (2015). Underlying Reasons Associated With Hospital Readmission Following Surgery in the United States. *JAMA*, 313(5), 483.
- Moellering, R. C. (2012). MRSA: the first half century. *Journal of Antimicrobial Chemotherapy*, 67(1), 4–11.
- Monecke, S., Berger-Bächi, B., Coombs, G., Holmes, A., Kay, I., Kearns, A., ... Ehricht, R. (2007). Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Panton-Valentine leukocidin. *Clinical Microbiology and Infection*, 13(3), 236–249.
- Morens, D. M., Taubenberger, J. K., & Fauci, A. S. (2008). Predominant Role of Bacterial Pneumonia as a Cause of Death in Pandemic Influenza: Implications for Pandemic Influenza Preparedness. *The Journal of Infectious Diseases*, 198(7), 962–970.

- Mpogoro, F. J., Mshana, S. E., Mirambo, M. M., Kidenya, B. R., Gumodoka, B., & Imirzalioglu, C. (2014). Incidence and predictors of surgical site infections following caesarean sections at Bugando Medical Centre, Mwanza, Tanzania. *Antimicrobial Resistance and Infection Control*, 3(1), 25.
- Mu, Y., Edwards, J. R., Horan, T. C., Berrios-Torres, S. I., & Fridkin, S. K. (2011). Improving Risk-Adjusted Measures of Surgical Site Infection for the National Healthcare Safety Network. *Infection Control & Hospital Epidemiology*, 32(10), 970–986.
- Müller-Premru, M., Strommenger, B., Alikadic, N., Witte, W., Friedrich, A. W., Seme, K., ... Gubina, M. (2005). New strains of community-acquired methicillin-resistant *Staphylococcus aureus* with Panton–Valentine leukocidin causing an outbreak of severe soft tissue infection in a football team. *European Journal of Clinical Microbiology & Infectious Diseases*, 24(12), 848–850.
- Mundhada, A., & Tenpe, S. (2015). A study of organisms causing surgical site infections and their antimicrobial susceptibility in a tertiary care Government Hospital. *Indian Journal of Pathology and Microbiology*, 58(2), 195.
- Naik, G., & Deshpande, S. R. (2011). A study on surgical site infections caused by *staphylococcus aureus* with a special search for methicillin-resistant isolates. *Journal of Clinical and Diagnostic Research*, 5(3), 502–508.
- Nejad, S. B., Allegranzi, B., Syed, S. B., Ellisc, B., & Pittet, D. (2011). Health-care-associated infection in Africa: a systematic review. *Bulletin of the World Health Organization*, 89, 757–765.
- Newman, M. J. (2009). Nosocomial and community acquired infections in Korle Bu Teaching Hospital, Accra. *West African Journal of Medicine*, 28(5), 300–303.
- O’Hara, F. P., Suaya, J. A., Ray, G. T., Baxter, R., Brown, M. L., Mera, R. M., ... AmrineMadsen, H. (2016). spa Typing and Multilocus Sequence Typing Show Comparable Performance in a Macroepidemiologic Study of *Staphylococcus aureus* in the United States. *Microbial Drug Resistance*, 22(1), 88–96.
- Odonkor, S. T., Newman, M. J., & Addo, K. K. (2012). Prevalence and antibiotic susceptibility profile of methicillin resistant *Staphylococcus aureus* in Accra, Ghana. *Microbiology Research*, 3(2), 20.
- Okon, K. O., Basset, P., Uba, A., Lin, J., Oyawoye, B., Shittu, A. O., & Blanc, D. S. (2009). Cooccurrence of predominant Panton-Valentine leukocidin-positive sequence type (ST) 152 and multidrug-resistant ST 241 *Staphylococcus aureus* clones in Nigerian hospitals. *Journal of Clinical Microbiology*, 47(9), 3000–3.
- Ostojić, M. (2008). Epidemiologic genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) by pulsed-field gel electrophoresis (PFGE). *Bosnian Journal of Basic Medical Sciences*, 8(3), 259–65.
- Owens, C. D., & Stoessel, K. (2008). Surgical site infections: epidemiology, microbiology and prevention. *Journal of Hospital Infection*, 70, 3–10.
- Park, K.-H., Greenwood-Quaintance, K. E., Uhl, J. R., Cunningham, S. A., Chia, N., Jeraldo, P. R., ... Patel, R. (2017). Molecular epidemiology of *Staphylococcus aureus* bacteremia

- in a single large Minnesota medical center in 2015 as assessed using MLST, core genome MLST and spa typing. *PLOS ONE*, 12(6), e0179003.
- Ranjan, K., Ranjan, N., & Gandhi, S. (2013). Surgical site infections with special reference to methicillin resistant *Staphylococcus aureus*: experience from a tertiary care referral hospital in North India. *International Journal of Research in Medical Sciences*, 1(2), 108.
- Rasigade, J.-P., Dumitrescu, O., & Lina, G. (2014). New epidemiology of *Staphylococcus aureus* infections. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 20(7), 587–8.
- Reygaert, W. C. (2013). Antimicrobial resistance mechanisms of *Staphylococcus aureus*. *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education*, 1, 297–305.
- Rodriguez, M., Hogan, P. G., Satola, S. W., Crispell, E., Wylie, T., Gao, H., ... Fritz, S. A. (2015). Discriminatory Indices of Typing Methods for Epidemiologic Analysis of Contemporary *Staphylococcus aureus* Strains. *Medicine*, 94(37), e1534.
- Ruimy, R., Maiga, A., Armand-Lefevre, L., Maiga, I., Diallo, A., Koumaré, A. K., ... Feil, E. J. (2008). The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. *Journal of Bacteriology*, 190(11), 3962–3968.
- Saadatian-Elahi, M., Teyssou, R., & Vanhems, P. (2008). *Staphylococcus aureus*, the major pathogen in orthopaedic and cardiac surgical site infections: A literature review. *International Journal of Surgery*. <https://doi.org/10.1016/j.ijsu.2007.05.001>
- Sabat, A. J., Budimir, A., Nashev, D., Sá-Leão, R., van Dijl, J. M., Laurent, F., ... Friedrich, A. W. (2013). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Eurosurveillance*, 18(4), 20380.
- Sakoulas, G., & Moellering, Jr., R. C. (2008). Increasing Antibiotic Resistance among Methicillin-Resistant *Staphylococcus aureus* Strains. *Clinical Infectious Diseases*, 46(S5), S360–S367.
- Salipante, S. J., SenGupta, D. J., Cummings, L. A., Land, T. A., Hoogestraat, D. R., & Cookson, B. T. (2015). Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology. *Journal of Clinical Microbiology*, 53(4), 1072–9.
- Sasaki, T., Tsubakishita, S., Tanaka, Y., Sakusabe, A., Ohtsuka, M., Hirotaki, S., ... Hiramatsu, K. (2010). Multiplex-PCR method for species identification of coagulase-positive staphylococci. *Journal of Clinical Microbiology*, 48(3), 765–9.
- Sasikumari, O., Sreekumary, P., & Jayalekha, B. (2016). The bacterial profile of surgical site infection occurring within one week after surgery in a tertiary care centre. *Journal of The Academy of Clinical Microbiologists*, 18(2), 86.
- Satta, G., Ling, C. L., Cunningham, E. S., McHugh, T. D., & Hopkins, S. (2013). Utility and limitations of Spa-typing in understanding the epidemiology of staphylococcus aureus bacteraemia isolates in a single University Hospital. *BMC Research Notes*, 6(1), 398.
- Schaumburg, F., Alabi, A. S., Peters, G., & Becker, K. (2014). New epidemiology of *Staphylococcus aureus* infection in Africa. *Clinical Microbiology and Infection*, 20(7), 589-596.

- Schaumburg, F., Köck, R., Friedrich, A. W., Soulanoudjingar, S., Ngoa, U. A., von Eiff, C., ... Becker, K. (2011). Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. *PLoS Neglected Tropical Diseases*, 5(5), e1150.
- Scott, R. D. (2009). The Direct medical costs of healthcare-associated infections in U.S. hospitals and the benefits of prevention. Retrieved March 13 2019 from [https://doi.org/http://www.cdc.gov/hai/pdfs/hai/scott\\_costpaper.pdf](https://doi.org/http://www.cdc.gov/hai/pdfs/hai/scott_costpaper.pdf)
- Seni, J., Bwanga, F., Najjuka, C. F., Makobore, P., Okee, M., Mshana, S. E., ... Kateete, D. P. (2013). Molecular Characterization of *Staphylococcus aureus* from Patients with Surgical Site Infections at Mulago Hospital in Kampala, Uganda. *PLoS ONE*, 8(6), e66153.
- Shittu, A. O., Okon, K., Adesida, S., Oyedara, O., Witte, W., Strommenger, B., ... Nübel, U. (2011). Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. *BMC Microbiology*, 11(1), 92.
- Siegel, J. D., Rhinehart, E., & Jackson, M. (2006). Management of Organisms In Healthcare Settings , 2006. *CDC Infection Control*, 2016(5 Arpil), 1–74.
- Singhal, N., Kumar, M., Kanaujia, P. K., & Viridi, J. S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, 6(2015), 791.
- Smyth, E. T. M., McIlvenny, G., Enstone, J. E., Emmerson, A. M., Humphreys, H., Fitzpatrick, F., ... Spencer, R. C. (2008). Four Country Healthcare Associated Infection Prevalence Survey 2006: overview of the results. *Journal of Hospital Infection*, 69(3), 230–248.
- Song, J. H., Hsueh, P. R., Chung, D. R., Ko, K. S., Kang, C. I., Peck, K. R., ... Hung Van, P. (2011). Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: An ANSORP study. *Journal of Antimicrobial Chemotherapy*, 66(5), 1061–1069.
- Song, X., Oetgen, M. E., Magge, S. N., Berger, J. T., & Shah, R. K. (2018). Utilization of a Dual Surveillance Program to Reduce Surgical-site Infections. *Pediatric Quality and Safety*, 3(6), e121.
- Strommenger, B., Kettlitz, C., Weniger, T., Harmsen, D., Friedrich, A. W., & Witte, W. (2006). Assignment of *Staphylococcus* isolates to groups by spa typing, SmaI macrorestriction analysis, and multilocus sequence typing. *Journal of Clinical Microbiology*, 44(7), 2533–40.
- Takesue, Y., Kusachi, S., Mikamo, H., Sato, J., Watanabe, A., Kiyota, H., ... Yanagihara, K. (2017). Antimicrobial susceptibility of pathogens isolated from surgical site infections in Japan: Comparison of data from nationwide surveillance studies conducted in 2010 and 2014–2015. *Journal of Infection and Chemotherapy*, 23(6), 339–348.
- Taye, M. (2005). Wound infection in Tikur Anbessa hospital, surgical department. *Ethiopian Medical Journal*, 43(3), 167–74.
- The Electives Network: 37 Military Hospital. (n.d.). Retrieved July 30, 2019, from <https://www.electives.net/hospital/5617/preview>
- Tong, S., Davis, J., ... E. E.-C. microbiology, & 2015, U. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28(3), 603–661.

- Tristan, A., Ferry, T., Durand, G., Dauwalder, O., Bes, M., Lina, G., ... Etienne, J. (2007). Virulence determinants in community and hospital methicillin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection*, 65, 105–109.
- Weller, T. M. A. (2000). Methicillin-resistant *Staphylococcus aureus* typing methods: which should be the international standard? *Journal of Hospital Infection*, 44(3), 160–172.
- Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., & Nouwen, J. L. (2005). The role of nasal carriage in *Staphylococcus aureus* infections. *The Lancet Infectious Diseases*, 5(12), 751–762.
- Zimlichman, E., Henderson, D., Tamir, O., Franz, C., Song, P., Yamin, C. K., ... Bates, D. W. (2013). Health care-associated infections: A Meta-analysis of costs and financial impact on the US health care system. *JAMA Internal Medicine*, 173(22), 2039–2046.

## APPENDIX I: Participant Consent Form



NAVAL MEDICAL RESEARCH CENTER

Office of Research Administration

### APPENDIX 6 (IRB). Consent Form, English Version

#### VOLUNTARY CONSENT TO PARTICIPATE

You have been asked to volunteer to participate in a research study entitled:

**Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Associated with Surgical Site Infections in a Military and Civilian Hospital in Ghana**

#### General Information about Research

Surgical site infections (SSIs) are common causes of hospital infections. Multi-drug resistant germs such as methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL) producing bacteria frequently cause SSIs. The germs are associated with prolong hospitalization and subsequently high health care cost because patients infected with such germs have limited antibiotic options for treatment. It is important to screen patients with SSI to detect the causative germs and determine the antibiotics they are susceptible or resistant to; this will help in the selection of suitable treatment. This project will study germs such as ESBL and MRSA found in SSIs.

If you agree to participate, samples from the site of infection after your surgery will be collected. These samples will be coded and taken to the laboratory for analysis. Your age, sex, ward of admission, operation type, period of hospitalization, co-morbidities and antimicrobial therapy will be obtained from your hospital records.

#### Possible Risks and Discomforts

The risks that this study may pose to you are minimal. A swab will be taken from the wound. This is a routine procedure that is often done in the hospital to determine what bacteria are causing a patient's infection. You may experience slight pain when the sample is being taken.

#### Possible Benefits

The results from this study will help in the selection of the appropriate treatment for the surgical site infection. The data generated will also contribute to effective surveillance measures in the hospital. However, there will be no direct benefit to you.

#### Confidentiality

Your sample will be coded. Any information you give us will only be used for this study and will remain confidential.

#### Voluntary Participation and Right to Leave the Research

Participation in this study is voluntary. You can decide not to participate if you are not comfortable with the procedure described above.

#### Alternatives to Participation

If you do not participate, there will be no penalty to you and you will still receive treatment and care as per the standard of care at this hospital. That care may or may not include some of the diagnostic tests you would receive in this study.

#### Contacts for Additional Information

Any questions concerning this study may be addressed to the Principal Investigators: Dr. Beverly Egyir, Noguchi Memorial Institute for Medical Research, Bacteriology Department, Legon, Tel: **233-208918099**, (email: [begyir@noguchi.ug.edu.gh](mailto:begyir@noguchi.ug.edu.gh)) and Dr Andrew Letizia, Tel: **233244333027**.

#### Your rights as a Participant

This research has been reviewed and approved by the Institutional Review Boards of Noguchi Memorial Institute for Medical Research (NMIMR-IRB), Korle-Bu Teaching Hospital and Naval Medical Research Unit Number Three. If you have any questions about your rights as a research participant, ethical aspects of this study or any problem related to protection of research volunteers you can contact the IRB Office at NMIMR between the hours of 8am-5pm through the landline 0302916438 or email addresses: [nirb@noguchi.mimcom.org](mailto:nirb@noguchi.mimcom.org). You may also contact the Human Protection Office in NMIMR, Accra, Ghana at 0302916438 or Dr. Ramiro Gutierrez, NMRC IRB Chair by calling +001-301-319-3193 or sending mail to [Ramiro.I.Gutierrez.mil@mail.mil](mailto:Ramiro.I.Gutierrez.mil@mail.mil), or the IRB office at 37 Military hospital on 024 3004247. Participants can contact the IRB office between the hours of 8:00am-3:00pm. You may also contact the IRB office at Korle Bu Teaching Hospital, Tel: +233- Version 2.0 January 12, 2018

|                    |                   |      |      |
|--------------------|-------------------|------|------|
| NAVY MEDICINE HRPP |                   |      |      |
| HRPP#              | NA0003            | 2016 | 0011 |
| Approval Date:     | 16 September 2018 |      |      |
| Expiration Date:   | 12 September 2019 |      |      |
| Verified By:       | AC                |      |      |





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**PARTICIPANT AGREEMENT FORM FOR DISPOSITION OF BODILY SAMPLES**

Please cross out the statement that does not apply

1. I **authorize** the use of all biological material (e.g. body fluids, aspirates, tissues, etc.) collected as part of this research study to NAMRU-3. You understand that these samples may be used for other testing as determined by NAMRU-3 but your name or any information that could link your identity to these samples will be removed.

Signature /Thumbprint of patient (circle one):

Date:

2. I **DO NOT authorize** the use of all biological material (e.g. body fluids, aspirates, tissues, etc.) collected as part of this research study to NAMRU-3.

Signature /Thumbprint of patient (circle one):

Date:

Version 2.0 January 12, 2018

|                           |                   |      |      |
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| <b>NAVY MEDICINE HRPP</b> |                   |      |      |
| HRPP#,                    | NAMRU3            | 2016 | 0011 |
| Approval Date:            | 16 September 2018 |      |      |
| Expiration Date:          | 12 September 2019 |      |      |
| Verified By:              | AC                |      |      |

## APPENDIX II: Parental Consent Form



NAVAL MEDICAL RESEARCH CENTER

Office of Research Administration

### PARENTAL CONSENT FORM

**Title:** Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Associated with Surgical Site Infections in a Military and Civilian Hospital in Ghana

**Principal Investigators:** Dr. Beverly Egyir and Dr. Andrew Letizia

**Address:** Noguchi Memorial Institute for Medical Research, Bacteriology Department, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana. P. O. Box LG 581, Legon, Ghana.

### General Information about Research

Surgical Site infections (SSIs) are common causes of hospital infections. Multi-drug resistant germs such as methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL) producing bacteria frequently cause SSI. The germs are associated with prolong hospitalization and subsequently high health care cost because patients infected with such germs have limited antibiotic options for treatment. It is important to screen patients with SSI to detect the causative germs and determine the antibiotic they are susceptible or resistant to; this will help in the selection of suitable treatment. This project will study germs such as ESBL and MRSA found in SSIs.

If you agree to allow your child to participate, samples from the site of infection after the surgery will be collected. These samples will be coded and taken to the laboratory for analysis. The hospital records of your child such as age, sex, and ward of admission, operation type, period of hospitalization, and antibiotics taken will be obtained from the hospital folder.

### Possible Benefits

The results from this study will help in the selection of the appropriate treatment for the surgical site infection. The data generated will also contribute to effective surveillance measures in the hospital. However, there will be no direct benefit to your child.

### Possible Risks and Discomforts

The risks that this study may pose to your child are minimal. A swab will be taken from the wound. This is a routine procedure that is often done in the hospital to determine what bacteria are causing a patient's infection. Your child may experience slight pain when the sample is being taken.

### Confidentiality

Your child's sample will be coded. Any information we obtained from his/her hospital records and sample analysis will remain confidential.

### Voluntary Participation and Right to Leave the Research

Participation in this study is voluntary. You can decide not to allow your child to participate if you are not comfortable with the procedure described above.

### Alternatives to Participation

If you decide not to allow your child to participate, there will be no penalty to your child and he/she will still receive treatment and care as per the standard of care at this hospital. That care may or may not include some of the diagnostic tests your child would receive in this study.

Version 2.0 January 12, 2018

|                    |                   |      |
|--------------------|-------------------|------|
| NAVY MEDICINE HRPP |                   |      |
| HRPP#: NABDU3      | 2016              | 0011 |
| Approval Date:     | 16 September 2016 |      |
| Expiration Date:   | 12 September 2019 |      |
| Verified By:       | AC                |      |





**NAVAL MEDICAL RESEARCH CENTER**

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**PARTICIPANT AGREEMENT FORM FOR DISPOSITION OF BODILY SAMPLES**

Please cross out the statement that does not apply

1. I **authorize** the use of all biological material (e.g. body fluids, aspirates, tissues, etc.) collected from my child as part of this research study. I understand that these samples may be used for other testing as determined by NAMRU-3 but my child's name or any information that could link my child's identity to these samples will be removed.

Signature /Thumbprint of parent or guardian (circle one):

Date:

2. I **DO NOT authorize** the use of all biological material (e.g. body fluids, aspirates, tissues etc.) collected from my child as part of this research study to NAMRU-3.

Signature /Thumbprint of parent or guardian (circle one):

Date:

Version 2.0 January 12, 2018

|                           |                   |      |      |
|---------------------------|-------------------|------|------|
| <b>NAVY MEDICINE HRPP</b> |                   |      |      |
| HRPP#:                    | NAMRU3            | 2016 | 0011 |
| Approval Date:            | 16 September 2018 |      |      |
| Expiration Date:          | 12 September 2019 |      |      |
| Verified By:              | AC                |      |      |

## APPENDIX III: Child Assent Form



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### CHILD ASSENT FORM

#### Introduction

Our names are Dr. Beverly Egyir and Dr. Andrew Letizia from the **Bacteriology Department** at **Noguchi Memorial Institute for Medical Research and the Naval Medical Research Unit Number THREE, Ghana detachment, respectively. We are** conducting a research study entitled: **"Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Associated with Surgical Site Infections in a Military and Civilian Hospital in Ghana"**

We are asking you to take part in this study we are trying to learn more about antibiotic resistant bacteria (germs) such as MRSA and ESBL positive bacteria, frequently found in surgical site infections that can occur after surgical operations.

#### General Information

Surgical site infections (SSIs) are common causes of hospital infections. Multi-drug resistant germs such as methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL) producing bacteria or germs that frequently cause SSIs. The germs are associated with prolong hospitalization and subsequently high health care cost because patients infected with such germs have limited antibiotic options for treatment. It is important to screen patients with SSI to detect the causative germs and determine the antibiotics they are susceptible or resistant to; this will help in the selection of suitable treatment. This project will study germs such as ESBL and MRSA found in SSIs.

If you agree to participate, samples from the site of infection after your surgery will be collected. These samples will be coded and taken to the laboratory for analysis. Your age, sex, ward of admission, operation type, period of hospitalization, co-morbidities and antimicrobial therapy will be obtained from your hospital records.

#### Possible Benefits

The results from this study will help in the selection of the appropriate treatment for the surgical site infection. The data generated will also contribute to effective surveillance measures in the hospital. However, there will be no direct benefit to you.

#### Possible Risks and Discomforts

The risks that this study may pose to you are minimal. A swab will be taken from the wound. This is a routine procedure that is often done in the hospital to determine what bacteria are causing a patient's infection. You may experience slight pain when the sample is being taken.

#### Voluntary Participation and Right to Leave the Research

Participation in this study is voluntary. You can decide not to participate if you are not comfortable with the procedure described above. No one will be angry with you if you do not want to participate.

#### Alternatives to Participation

If you do not participate, there will be no penalty to you and you will still receive treatment and care as per the standard of care at this hospital. That care may or may not include some of the diagnostic tests you would receive in this study.

#### Confidentiality

Your sample will be coded. Any information you give us will only be used for this study and will remain confidential.

#### Contacts for Additional Information

You may ask us any questions about this study. You can contact Dr. Beverly Egyir, at **233-208918099** or Dr Andrew Letizia at **233-244333027** or talk to us the next time you see us.

Please talk about this study with your parents before you decide whether or not to participate. I will also ask permission from your parents before I take your sample. Even if your parents say "yes" you can still decide not to participate.

Version 2.0 January 12, 2018

|                    |                   |      |
|--------------------|-------------------|------|
| NAVY MEDICINE HRPP |                   |      |
| HRPP#: NAMB03      | 2016              | 0011 |
| Approval Date:     | 16 September 2016 |      |
| Expiration Date:   | 12 September 2019 |      |
| Verified By:       | AC                |      |

**Your rights as a Participant**

This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: [nirb@noguchi.mimcom.org](mailto:nirb@noguchi.mimcom.org). You may also contact the Human Protection Office in NMIMR, Accra, Ghana at 0302916438 or Dr. Ramiro Gutierrez, NMRC IRB Chair by calling +001-301-319-3193 or sending mail to [Ramiro.I.Gutierrez.mil@mail.mil](mailto:Ramiro.I.Gutierrez.mil@mail.mil). Any questions concerning this study may be addressed to: IRB office at 37 Military hospital on 024 3004247. Participant can contact the IRB office between the hours of 8:00am-3:00pm. Any questions concerning this study may be addressed to: IRB office at Korle Bu Teaching Hospital, Tel: +233-302666766 between the office hours of 9:00am-4:00pm or via email: [rdo@kbth.gov.gh](mailto:rdo@kbth.gov.gh)

**VOLUNTARY AGREEMENT**

By making a mark or thumb printing below, it means that you understand and know the issues concerning this research study. If you do not want to participate in this study, please do not sign this assent form. You and your parents will be given a copy of this form after you have signed it.

This assent form which describes the benefits, risks and procedures for the research titled *[Prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Associated with Surgical Site Infections in a Military and Civilian Hospital in Ghana]* has been read and or explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate.

Child's Name:.....

Researcher's Name:.....

Child's Mark/Thumbprint:.....

Researcher's Signature:.....

Date:.....

Date:.....

|                    |                   |      |
|--------------------|-------------------|------|
| NAVY MEDICINE HRPP |                   |      |
| HRPP#: NAKRU3      | 2016              | 0011 |
| Approval Date:     | 16 September 2018 |      |
| Expiration Date:   | 12 September 2019 |      |
| Verified By:       | AG                |      |



## APPENDIX IV: Case Report Form



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APPENDIX 8 (IRB). Case Report Form, English Version.

### Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamase (ESBL) Producing Bacteria Associated with Surgical Site Infections in a Military and Civilian Hospital in Ghana

1. Subject ID.....
2. Gender..... Age..... Department/ Ward.....
3. Diagnosis.....
4. Type of surgical Operation.....
5. Date of operation.....
6. Duration of Operation.....
7. Type of infection..... a) Superficial incisional, b) Deep incisional c) Organ/space  
d) Unknown
8. Date of onset of infection.....
9. Antibiotic therapy before operation.....
10. Antibiotic therapy after operation.....
11. Period of hospitalization.....
12. Co-morbidities.....
13. Days post operation.....

## APPENDIX V: NHSN Operative Procedures Followed for 30 and 90 Days

### Surveillance Periods for SSI Following Selected Operative Procedure Categories

| 30-day Surveillance |                                                                       |          |                                    |
|---------------------|-----------------------------------------------------------------------|----------|------------------------------------|
| Category            | Operative Procedure                                                   | Category | Operative Procedure                |
| AAA                 | Abdominal aortic aneurysm repair                                      | LAM      | Laminectomy                        |
| AMP                 | Limb amputation                                                       | LTP      | Liver transplant                   |
| APPY                | Appendix surgery                                                      | NECK     | Neck surgery                       |
| AVSD                | Shunt for dialysis                                                    | NEPH     | Kidney surgery                     |
| BILI                | Bile duct, liver, or pancreatic surgery                               | OVRY     | Ovarian surgery                    |
| CEA                 | Carotid Endarterectomy                                                | PRST     | Prostate surgery                   |
| CHOL                | Gallbladder surgery                                                   | REC      | Rectal surgery                     |
| COLO                | Colon surgery                                                         | SB       | Small bowel surgery                |
| CSEC                | Caesarean section                                                     | SPLE     | Spleen surgery                     |
| GAST                | Gastric surgery                                                       | THOR     | Thoracic surgery                   |
| HTP                 | Heart transplant                                                      | THYR     | Thyroid and/or parathyroid surgery |
| HYST                | Abdominal hysterectomy                                                | VHYS     | Vaginal hysterectomy               |
| KTP                 | Kidney transplant                                                     | XLAP     | Exploratory laparotomy             |
| 90-day Surveillance |                                                                       |          |                                    |
| Category            | Operative Procedure                                                   | Category | Operative Procedure                |
| BRST                | Breast surgery                                                        | HER      | Herniorrhaphy                      |
| CARD                | Cardiac surgery                                                       | HPRO     | Hip prosthesis                     |
| CBGB                | Coronary artery bypass graft with both chest and donor site incisions | KPRO     | Knee prosthesis                    |
| CBGC                | Coronary artery bypass graft with chest incision only                 | PACE     | Pacemaker surgery                  |
| CRAN                | Craniotomy                                                            | PVBY     | Peripheral vascular bypass surgery |
| FUSN                | Spinal fusion                                                         | VSHN     | Ventricular shunt                  |
| FX                  | Open reduction of fracture                                            |          |                                    |

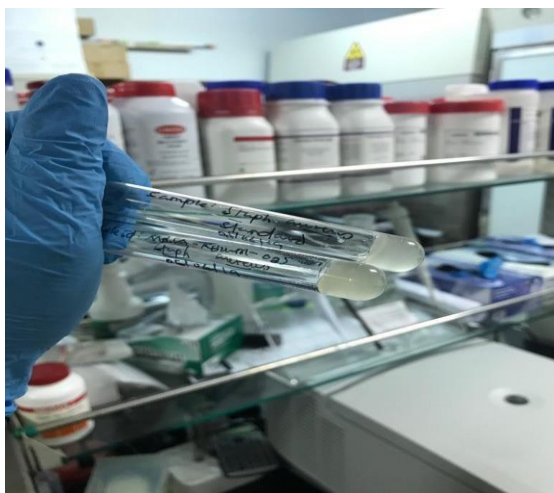
**APPENDIX VI: DNA Quantification for NGS Using the Qubit Double Strand (ds)  
High Sensitivity (HS) Kit**

| <b>Sample ID</b> | <b>DNA Concentration (ng/μl)</b> |
|------------------|----------------------------------|
| MRSA-37MH-004    | 6.99                             |
| MRSA-37MH-019    | 39.5                             |
| MRSA-37MH-031    | 2.82                             |
| MRSA-37MH-038    | 37.3                             |
| MRSA-37MH-060    | 0.66                             |
| MRSA-37MH-063    | 38.3                             |
| MRSA-37MH-065    | 13.4                             |
| MRSA-37MH-066    | 8.89                             |
| MRSA-KBU-M-033   | 10.8                             |
| MRSA-KBU-M-035   | 2.02                             |
| MRSA-KBU-M-037   | 4.42                             |
| MRSA-KBU-S-005   | 1.0                              |
| MRSA-KBU-M-045   | 21.3                             |

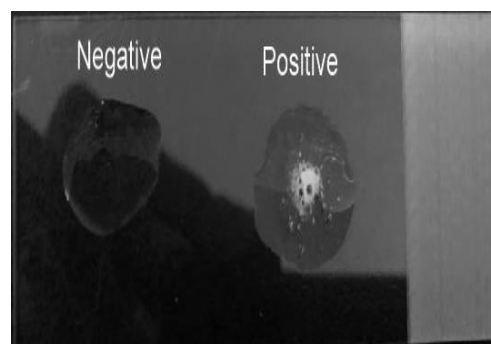
## APPENDIX VII: Tests for Identification and Susceptibility testing of *S. aureus*



Isolates on Mannitol Salt Agar (Left) and Blood agar (Right)



Coagulase Testing. Control (Top),  
Positive Sample (Bottom)



Catalase Test for *S. aureus* Isolates.

## Vancomycin Testing for *S. aureus* Isolates

