UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

"NASAL COLONIZATION WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AMONG SICKLE CELL DISEASE CHILDREN"

BY

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DECLARATION

I, Vera Ama Appiah, declare that the work presented in this thesis is the result of my own research carried out in the Department of "Medical Microbiology" Research Laboratory, "School of Biomedical and Allied Health Sciences" (SBAHS), Korle Bu, under the supervision of Prof. Eric Sampane-Donkor (SBAHS) and Dr. George Antepem Pesewu (SBAHS), and that all "references cited in this work have been duly acknowledged".

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DEDICATION

I dedicate this work to my husband, kids, and friends.

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I appreciate the contribution that the following individuals made to my postgraduate training and the success of this research: my supervisors (Prof. Eric Sampane-Donkor of the Department of Medical Microbiology and Dr. George Antepem Pesewu), both of the; Dr. Samuel Duodu of the Department of Biochemistry, Cell and Molecular Biology/WACCBIP; Mr. Nana Boakye Alahaman, as well as the technical and administrative staff of the departments of Medical Microbiology and Medical Laboratory Sciences.

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a public health threat as it is extensively resistant to antimicrobials, is associated with persistent outbreaks in hospital and community settings, and is associated with markedly increased healthcare costs. Moreover, sickle cell disease patients frequently visit hospitals and often consume antibiotics, hence could potentially harbor MRSA, and consequently serve as reservoirs for dissemination of the pathogen. Globally, little is known about MRSA carriage among sickle cell disease patients.

General aim: The aim of this study was to investigate nasal carriage of *S. aureus* and MRSA among children with and without sickle cell disease in Accra, including the prevalence, risk factors and antibiogram.

Methodology: "This study was cross-sectional, and involved 120 children with sickle cell disease and 100 apparently healthy children without the disease recruited at the Princess Marie Louis Children's Hospital (PML) in Accra. 'Nasal swab specimens were collected from the study participants and cultured. *S. aureus* isolates were confirmed by the tube coagulase test, while MRSA was confirmed via PCR targeting the *mec*A gene. *S. aureus* susceptibility to standard antimicrobial agents was tested by the Kirby Bauer method. A pretested structured questionnaire was also used to obtain data on demographic, household, and clinical features of the study participants. A binary logistic regression was used to identify determinants of *S. aureus* and MRSA carriage among the study participants.

Results: The nasal carriage prevalence of *S. aureus* were 33.3% (n = 40) and 10% (n = 10) respectively among the sickle cell disease children and the participants of the control group. With regard to MRSA nasal carriage prevalence, the respective values were 9.2% (n = 11) and 5% (n = 5). Sickle cell disease was significantly associated with *S. aureus* colonization (χ^2 = 16.91, p < .0001, OR = 4.045), but not MRSA colonization (χ^2 = 1.4, p = .237). The significant predictors of *S. aureus* colonization among the sickle cell disease patients were increasing age (p = 0.003; OR = 1.275), male gender (p = 0.018; OR = 0.344), living in self-contained apartments (p = 0.033; OR = 3.632), and practice of self-medication (p = 0.039; OR = 0.233). In the control group, history of hospitalization in the past year was a risk factor for carriage of both *S. aureus* (p = 0.048; OR = 14.333) and MRSA (p = 0.044; OR = 21.176). The proportion of *S. aureus*

isolates that were multidrug resistant (MDR) was 92.5% (37/40) in the sickle cell group and 100% (10/10) in the control group.

Conclusions: Among children in Accra, sickle cell disease predisposed to carriage of *S. aureus*, but not MRSA; the odds ratio of S. *aureus* carriage in relation to the presence of sickle cell disease was 4.045. In addition, increasing age, male gender, living in self-contained apartments, and practice of self-medication, were risk factors for carriage of *S. aureus* among the sickle cell disease children. History of hospitalization in the past year, was a risk factor for MRSA and S. aureus carriage. Finally, both the sickle cell disease children and participants of the control group harbored multidrug resistant *S. aureus*, and this may be due to extensive antimicrobial use in the country.

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LIST OF ABBREVIATIONS

"ATCC…….American Type Culture Collection"

"BMI……...…….Body mass index"

CA-MRSA……Community-associated MRSA

"CI……...…….Confidence interval"

CONS……Coagulase-negative Staphylococci

CO_2……Carbon dioxide

DNA……..…….Deoxyribonucleic acid

dNTP……...Deoxyribonucleoside triphosphate

EPRC……..…Ethical and Protocol Review Committee

HA-MRSA… ……....…Healthcare-associated MRSA

KBTH……..…… Korle Bu Teaching Hospital

"LA-MRSA…….….. Livestock-associated MRSA"

MDR……...…….Multidrug resistant

$MgCl_2$……Magnesium chloride

"MSSA……..…… Methicillin-susceptible *Staphylococcus aureus"*

"MRSA…….Methicillin-resistant *Staphylococcus aureus"*

N/A……...……..Not applicable

“OR……Odds ratio”

“PCR……..…...Polymerase chain reaction”

PML……..…...Princess Marie Louis Children’s Hospital

PVL……..Panton-Valentine leucocidin

Res…….……..Resistant

RNase……...Ribonuclease

SSTI…….Skin and soft tissue infection

TB……..…Tuberculosis

°C……...…….Degree Celsius

χ2……..……Chi-square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

"Staphylococcus aureus (*S. aureus*), a ubiquitous human commensal, is one of the most frequently isolated Gram positive bacteria in clinical specimens (Fowler Jr. *et al*., 2003; Crowley *et al*., 2008; Donkor *et al*., 2010; Paulsen *et al*., 201), and possesses the propensity of developing resistance to a multitude of antibiotics (Shenoy *et al*., 2014)". The predisposition to antibiotic resistance in *S. aureus* has most likely been reinforced by extensive antibiotic usage. "The most important phenomenon of antimicrobial resistance in *S. aureus* is probably the development of methicillin resistance". "This phenomenon has contributed to the evolvement of *S. aureus* from a commensal to a very important pathogen that poses a serious public health threat (Elixhauser & Steiner, 2007; Klevens *et al*., 2007)". *S. aureus* is a commensal of various parts of the body, including the skin, perineum and pharynx, but preferentially colonizes the moist squamous epithelium of the anterior nares (Kluytmans *et al.*, 1995; Foster, 2004; Wertheim *et al.*, 2005). Various studies have demonstrated that *S. aureus* carriage precedes its infection. For instance, *S. aureus* blood isolates from individuals diagnosed with bacteremia have been shown to be identical to corresponding isolates from the anterior nares (Von Eiff *et al*., 2001). Moreover, there is evidence from several studies that nasal decolonization of the pathogen reduces the incidence of its infections (Yu *et al*., 1986; Chow & Yu, 1989; Holton *et al*., 1991; Boelaert *et al*., 1993; Wenzel & Perl, 1995; Kluytmans *et al*., 1996).

"MRSA strains emerged soon after the use of methicillin in clinical practice (Barber *et al*., 1961)". "They primarily were associated with healthcare facilities (causing nosocomial

infections), but are now recognized as key culprits in community infections, and strains with such an attribute have been referred to as community associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) (David *et al.*, 2010; DeLeo *et al*., 2010)". MRSA transmission occurs through exposure to infected persons, usually, by skin to skin contact (Herman*et al*., 2008). "Methicillin resistance results from the acquisition of an extra 20-50kb chromosomal DNA called the *mec*A gene, which can be found on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*)". This acquired gene codes for a new penicillin binding protein, *PBP 2a*, which has a reduced affinity for methicillin (Fuda *et al*., 2004). The acquisition of the gene further confers on the organism resistance to all βlactam antibiotics, including penicillin, methicillin, and other penicillin derivatives. Resistance of MRSA strains to erythromycin (macrolides), ciprofloxacin (Quinolones), cephalosporins, vancomycin (aminoglycosides), linezolid and daptomycin have also been reported (Archer *et al*., 1998).

"Sickle cell disease (SCD) refers to a group of red blood cell disorders, resulting from abnormalities in haemoglobin structure, leading to the development of sickle-shaped red blood cells (Stuart *et al*., 2004)". A point mutation involving the substitution of adenine for thymine in the β-globin gene (borne on chromosome 11) results in a GTG codon, instead of the wildtype GAG codon. The resultant GTG codon codes for valine, instead of the wildtype glutamate, and this is borne on the surface of the mutant protein. This genetic defect results in the change in haemoglobin structure (Rees *et al*., 2010). "Individuals with HbSS form of SCD inherit a sickle cell gene (S) from each parent (Creary*et al*., 2007)". In comparison to the rest of the world, Sub-Saharan Africa accounts for a higher burden of SCD, recording a 75% prevalence (Grosse *et al.*, 2011). The immunocompromised state of individuals with SCD predisposes them to infections. However, the frequent use of antibiotics as prophylaxis, persistent illness, and longer hospital

stays by HbSS children may precipitate the development of antibiotic resistance, thus increase susceptibility to MRSA carriage and infections. Of much interest is the role of MRSA in morbidity and mortality in HbSS children (Booth *et al.,* 2009). It is therefore important that studies are carried out in different geographical settings of interest to determine the carriage of MRSA in HbSS individuals. This may provide findings of clinical relevance aimed at the formulation of therapeutic policies for HbSS patients. "Penicillin and cotrimoxazole prophylaxis among SCD and HIV/AIDS patients, respectively, have been challenged with the emergence and spread of multidrug resistant pneumococcal strains on the global scene (Soeters *et al*., 2012; Dayie *et al.,* 2015)."

1.2 Problem statement

Over the years, the proportion of isolation of MRSA among *S. aureus* infections has increased to as high as 70% (Hsueh *et al*., 2002; NNIS, 2004; Tiemersma *et al*., 2004; Amaral *et al*., 2005; EARSS, 2006). In the United States, in the year 2014, it accounted for an estimated 72,444 invasive infections, resulting in 9,194 deaths (CDC, 2014). Inpatient stays due to MRSA infections cost $14,000, relative to the financial burden for all other stays ($7,600), with a two-fold increase in the length of hospitalization (Elixhauser & Steiner, 2007; Klevens *et al*., 2007).

Owing to the impairment of their immune system, sickle cell disease patients frequently patronize healthcare services and consume antibiotics. Though this does not constitute irrational antibiotic use, it could invariably select for antibiotic resistant pathogens among these individuals, and potentially make them reservoirs for multidrug resistant commensal and pathogenic microbes, such as MRSA. MRSA carriage is an antecedent to subsequent infections (Pathak *et al*., 2010; Ruhe *et al*., 2011; Seybold *et al*., 2011; Kutlu *et al*., 2012), and its infections

in sickle cell disease patients could further challenge the health of these individuals. Surveillance studies in the country have reported rates of MRSA carriage to range between 0.3–33% (Donkor & Nartey, 2007; Odonkor *et al*., 2012; Egyir *et al*., 2014a; Egyir *et al*., 2014b; Egyir *et al*., 2016; Donkor *et al*., 2019), although this does not include information on sickle cell disease patients. Since 2012, MRSA has caused several outbreaks in the country (Donkor *et al*., 2018). A replication of these outbreaks in sickle cell disease patients could be fatal. The public health threat that MRSA could pose to this immunologically challenged sickle cell disease patients, warrants investigation and surveillance in this at risk population.

1.3 Justification of the study

Notwithstanding the fact that sickle cell disease patients could potentially harbour MRSA at high rates, this risk group has received little attention from researchers studying MRSA carriage. "Microbiology researchers focusing on sickle cell disease patients have usually focused on the occurrence of *Streptococcus pneumoniae* in them (Kateete *et al*., 2012; Dayie *et al*., 2018)". Yet, among sickle cell disease (SCD) patients, *S. aureus* holds more potential in causing invasive diseases than *S. pneumoniae* (Okuonghae et al., 1993; Akuse et al., 1996; Aken'ova et al., 1998; Thanni, 2006; Kizito et al., 2007). Moreover, MRSA carriage studies seem to have focused on the general population (Donkor *et al*., 2007; Egyir *et al*., 2013; Egyir *et al*., 2014; Eibach *et al*., 2017) and a few other risk groups, such as persons with HIV infection (Sampane-Donkor *et al*., 2017; Egyir *et al*., 2016; Donkor *et al*., 2019). Hence knowledge on the epidemiology of MRSA, including carriage rates, determinants of carriage, and antibiotic resistance of colonizing strains, in relation to sickle cell disease patients is limited. Therefore, conducting MRSA surveillance studies among these individuals would provide additional insights about the epidemiology of the pathogen, and provide data to guide the management of its infections in this at risk population.

1.4 General aim

The aim of the study was to investigate *S. aureus* and MRSA colonization among children with sickle cell disease and those without the disease attending the outpatient clinic of the Princess Marie Louis Children's Hospital (PML).

1.5 Specific objectives

The specific objectives of this study were:

- To determine whether sickle cell disease predisposes children to *S. aureus* and MRSA colonization
- To determine the risk factors for colonization with *S. aureus* and MRSA among the sickle cell disease patients
- To determine the antimicrobial resistance patterns of the *S. aureus* and MRSA isolates of the study participants

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Staphylococcus aureus*: its characteristics and infections

S. aureus is a Gram positive, aerobic, non-motile and non-sporulating pus producing coccus (Deurenberg et al., 2009). When viewed microscopically, *S. aureus* appears as 0.5 to 1.5 µm balls clumped in grapelike clusters with more than 200 strains (Dissemond et al., 2009). Presentations of *S. aureus* infections include the following: skin and soft infections, scalded and skin syndrome, folliculitis, furuncle, carbuncle, bone infections toxic shock syndrome, endocarditis arthritis, pneumonia and deep tissue abscess infections (Klevens et al., 2007).

2.2 Resistance of *Staphylococcus aureus* to antibiotics

Ever since the 1940s, *Staphylococcus aureus* strains resistant to the antibiotic penicillin have been reported (Lowy, 2003; Peacock & Paterson, 2015). This is known to be as a result of expression of beta lactamase by the organism; this hydrolyzes the beta lactam ring of the penicillin molecule (Massova & Mobashery, 1998; Lowy, 2003; Peacock & Paterson, 2015). Yet, the most significant phenomenon of antimicrobial resistance in the organism is probably the emergence of methicillin resistance, as it confers resistance to all beta-lactam antibiotics, and complicates the management of *S. aureus* infections through its association with higher periods of hospitalizations and increased healthcare costs (Klevens *et al*., 2007; De Kraker *et al*., 2011). These strains are reservoirs of the mec genes, which are carried on different homologues of the Staphylococcal cassette chromosome (SCCmec) (Milheiriço, Oliveira, & de Lencastre, 2007; Jensen & Lyon, 2009; Malachowa & DeLeo, 2010). It is the SCCmec-carried cassette

chromosome recombinases (ccrA/ccrB or ccrC) carried that facilitate mec gene excision and integration, and together with the mec genes, provide a basis for SCCmec characterization (Jemili-Ben, Boutiba-Ben, & Ben, 2006; Ramdani, et al., 2006). Following the evolution of MRSA, methicillin resistance in *S. aureus* had been attributed to carriage of the mecA gene until the recent discovery of the distinct mecA homologue, the mecC gene, primarily identified in a single European MRSA lineage (Shore *et al*., 2011; Becker *et al*., 2014; Paterson, Harrison, & Holmes, 2014). The mecA gene, the primary methicillin resistance mediator, encodes a penicillin binding protein alien to *S. aureus*, PBP2' or PBP2a, which has a reduced affinity for methicillin (Hartman & Tomasz, 1984; Utsui & Yokota, 1985; Chambers, 1997; Peacock & Paterson, 2015; Fisher & Mobashery, 2016).

MRSA strains were initially limited to healthcare settings, and were referred to as healthcare-associated MRSA (HA-MRSA) (Chambers & Deleo, 2009; DeLeo *et al*., 2010; Rudkin *et al*., 2012). Overtime, though, MRSA strains called community-associated MRSA (CA-MRSA), which are characteristically distinct from HA-MRSA strains, by virtue of their having lower propensity for multidrug resistance and smaller SCCmec cassettes than HA-MRSA strains, emerged in communities (Chambers & Deleo, 2009; DeLeo *et al*., 2010; Malachowa & DeLeo, 2010; Rudkin *et al*., 2012). These strains discovered later are also more virulent, more adapted to the human skin, and mostly warranting hospital admission due to serious skin and soft tissue infections in apparently healthy individuals, whereas the former is more associated with wound infections and systemic infections (Chambers & Deleo, 2009; DeLeo *et al*., 2010; Thurlow *et al*., 2013). 'Yet, another lineage of MRSA strains that are adapted to livestock, called livestock-associated MRSA (LA-MRSA), has also been identified (Quitcoco *et al*., 2013)'.

The development of penicillin resistance, and subsequently methicillin resistance, necessitated modifications in treatment options for *S. aureus* therapy; yet there has been an emergence of resistance to these newer therapeutic options too, probably due to selective pressure. In the case of the newer beta-lactams, ceftaroline and ceftrobiprole, for instance, the resistance has been attributed to *S. aureus* strains bearing newer PBP2a mutants (Chan, et al., 2015; Kelley, Jousselin, Barras, Lelong, & Renzoni, 2015; Strommenger, Layer, Klare, & Werner, 2015). However ceftaroline resistance cannot be exclusively attributed to ceftaroline usage, as in a recent study conducted by Egyir *et al*. (2015) in Ghana, where ceftaroline is not widely used, 20% of the MRSA strains isolated, which belonged to the ST247 clone, were ceftaroline resistant, hence suggesting a mechanism of ceftaroline resistance in MRSA which could probably be linked to factors other than ceftaroline usage. This is probably the case as McKinnell *et al*. (2012) have documented evidence suggesting that prior use of ceftriaxone (1 month), but not of vancomycin, is associated with bloodstream infections of vancomycin-resistant Enterococci (VRE). This observation is supported by studies which have reported a reduction in infections by VRE when broad-spectrum use of cephalosporins is kept at a minimum (May *et al*., 2000).

Besides these, *S. aureus* has developed resistance against several other antibiotics, to varying degrees. In one drug of choice for treating MRSA infections, linezolid, for instance, resistance is rare, and the few cases of resistance have usually been associated with loss of resistance to erythromycin, presence of the plasmid-borne *cfr* gene, as well as mutations in the L3 and L4 ribosomal proteins arising from substitution of amino acids (Howe *et al*., 2002; Morales *et al*., 2010; Long & Vester, 2012; Shore *et al*., 2016). Similar to the case of linezolid resistance, resistance to daptomycin, another drug of choice for MRSA therapy, is rare; resistance occurs

during prolonged therapy, is potentiated by previous vancomycin therapy, and is attributed to mutants of the genes responsible for activating bacterial defenses during cell wall damage (Arbeit *et al*., 2004; Bayer, Schneider, & Sahl, 2013; Miller, Bayer, & Arias, 2016). Perhaps, daptomycin resistance is one phenomenon of resistance in *S. aureus* that may be looked upon somewhat favorably, as it confers beta-lactam antibiotic susceptibility on MRSA strains (Renzoni *et al*., 2017). This phenomenon has offered a therapeutic option for the treatment of daptomycin-resistant MRSA infections – treatment with an amalgam of beta-lactam antibiotics and daptomycin (Dhand *et al*., 2011; Dhand & Sakoulas, 2014; Sakoulas, Moise, & Casapao, 2014). That prior vancomycin therapy accentuates daptomycin resistance is further supported by the fact that VISA strains with no prior exposure to daptomycin have demonstrated daptomycin MICs higher than the regular breakpoints of susceptibility for daptomycin, demonstrating a clear relationship between the two antibiotics (Allington & Rivey, 2001; Howden *et al*., 2010, 2011). Furthermore, although the synergistic effect of beta lactams-daptomycin complexes on daptomycin resistant *S aureus* strains is yet to be observed for vancomycin, genes such as *dtl*, *vraSR*, *walKR*, and *rpoC*, whose mutations are associated with the emerging, though sparingly encountered, strains resistant to vancomycin –vancomycin-intermediate *Staphylococcus aureus* (VISA) – have been found in daptomycin resistant strains too (Foster, 2017). Notwithstanding the fact that these emerging VISA strains have not spread to reach clinically significant levels, their occurrence is a matter of growing concern, as vancomycin is the mainstay of MRSA therapy (Courvalin, 2006; Gardete & Tomasz, 2014). The VISA strains are preceded by subpopulations called hetero-vancomycin-intermediate *Staphylococcus aureus* (h-VISA), usually develop during prolonged therapy as occurs with daptomycin, and have been demonstrated to have significantly higher minimum inhibitory concentrations (MICs) in comparison with strains

that are sensitive to the antibiotic (4 – 8 µg/ml vs ≤ 2 µg/ml) (Courvalin, 2006; Gardete & Tomasz, 2014). The proposed mechanism through which VISA and h-VISA are refractory to vancomycin therapy is that these strains modify their cell wall structure and become more thickened, hence forming a less penetrable barrier for the drug to cross and reach its target (Howden *et al.*, 2010; Gardete & Tomasz, 2014). With increasing proportions of *S. aureus* strains that are methicillin resistant, the biggest threat to *S. aureus* therapy could be the potential for the spread of vancomycin resistance, which is highly probable, given the high content of the transmissible vancomycin resistance genes, the *van* genes, in the enterococci (Courvalin, 2006; Donkor & Nartey, 2007; Odonkor *et al*., 2012; Zeng *et al*., 2016; Karikari *et al*., 2017).

Some other antibiotics to which *S. aureus* strains have displayed resistance include co-trimoxazole (Donkor *et al*., 2019), fusidic acid (O'Neill & Chopra, 2006), rifampicin (Aubry-Damon, Soussy, & Courvalin, 1998), aminoglycosides (Ramirez & Tolmasky, 2010), streptogramins (Yu *et al*., 2014), mupirocin (Foster, 2017), and fluoroquinolones (Hooper & Jacoby, 2015). Co-trimoxazole, an amalgam of the sulphonamide antibiotic sulfamethoxazole and trimethoprim, is used in empirical therapy of '*S. aureus* skin and soft tissue infections' (Wormser, Keusch, & Heel, 1982; Goldberg & Bishara, 2012). Resistance to this antibiotic is commonly encountered in African *S. aureus* isolates, as is indicated by several recent reports emanating from the region (Aiken *et al*., 2014; Nurjadi *et al*., 2014; Pesewu *et al*., 2014; Egyir *et al*., 2016; Donkor *et al*., 2019); the situation is no different from Asia (Nurjadi *et al*., 2015), but is rare in Europe (den Heijer *et al*., 2013).

Similarly, in both rifampicin and fusidic acid, treatment failure and resistance facilitation through single step high level mutations occur in monotherapy, but administering the two antibiotics synergistically offers an effective option for MRSA therapy (Foster, 2017). Resistance to fusidic

acid has primarily been attributed to *S. aureus* expression of the mobile genetic element-harbored *FusB* and *FusC* genes, and occasionally, *FusA* mutations (Nagaev *et al*., 2001; O'Neill & Chopra, 2006; Fernandes, 2016). In the case of rifampicin, it is as a result of *S. aureus* strains in which rifampicin binding sites, as well as regions proximal to these sites have undergone mutations such that these sites have lesser affinity for rifampicin (Aubry-Damon, Soussy, & Courvalin, 1998; Wichelhaus *et al*., 1999).

As regards aminoglycosides, such as gentamycin and neomycin, resistance by *S. aureus* is as a result of acquisition of mobile genetic elements whose encoded enzymes modify aminoglycosides (Jensen & Lyon, 2009; Ramirez & Tolmasky, 2010). Resistance to streptogramins has mainly been observed in synercid, in both human and animal *S. aureus* pathogens (Argudin *et al*., 2011; Yu *et al*., 2014; Shore *et al*., 2016).

With regard to mupirocin, the *S. aureus* nasal decolonization agent, resistance results from target site mutations and *MupA*-harboring plasmid acquisition, which respectively specify low- and high-level resistance (Gordon *et al*., 2010; Foster, 2017). Mutations that reduce drug affinity to target have also been implicated in fluoroquinolone resistance – mutations in the topoisomerases – together with the characterized inherent pumps, *NorA*, *NorB*, and *NorC*, which pump out the antibiotics to achieve sub-therapeutic levels (Yu, Grinius, & Hooper, 2002; Truong-Bolduc *et al*., 2005; Truong-Bolduc, Strahilevitz, & Hooper, 2006; Hooper & Jacoby, 2015).

2.3 MRSA epidemiology

Various MRSA strains have been reported with increasing frequency worldwide. Initially, MRSA was thought to be confined to hospital settings (HA-MRSA) (Chambers & Deleo, 2009;

Rudkin *et al*., 2012), until the emergence of community-associated MRSA in population groups lacking health-care setting related risk factors (Baggett *et al*., 2003; Buckingham *et al*., 2004). The distinction and specificity in continental distribution of CA-MRSA and HA-MRSA has been bridged (Naimi *et al*., 2001; Duffour *et al*., 2002; O'Brien *et al*., 2004; Wang *et al*., 2004). For example the strain USA300, previously restricted to the United States of America (Liu *et al*., 2008; King *et al*., 2006), has been reported in Singapore (Hsu *et al*., 2005) and many other countries (Shibuya *et al*., 2008), including Ghana (Egyir *et al*., 2015). Almost a quarter of a century elapsed between the emergence of MRSA in industrialized countries in the 1960s and its first description in Africa in 1988 (Peddle *et al*., 1988). Various studies across the globe have reported varying carriage prevalence of MRSA: 12% in the USA (Rabinowitz*et al*., 2012), 7.3% in Italy, 8.1% in Spain, 7.1% in Israel, and 14.6% in France (Bilavsky*et al*., 2012). With regard to sub-Saharan Africa, there is paucity of data regarding the epidemiology of MRSA. One study on the epidemiology of MRSA lineages in five major African towns reported a prevalence of 15% (Breurec *et al*., 2011). Studies from a number of hospitals in Kenya have shown that about 90% of patients in the burns unit were colonized with MRSA (Muthotho *et al*., 1995). Moreover, a prevalence of 7 % was reported in Madagascar, and 82 % in a study emanating from Egypt (Abdulgader *et al*., 2015). In Ghana, MRSA prevalence of 0–34.8% has been reported by various studies (Donkor & Nartey, 2007; Odonkor*et al*., 2012; Egyir *et al*., 2014a; Egyir *et al*., 2014b; Egyir *et al*., 2016; Karikari *et al*., 2017). The high prevalence of 15 to 34.8% reported by some of these studies (Donkor & Nartey, 2007; Odonkor *et al*., 2012; Karikari *et al*., 2017) may be due to variations in the methodological approaches to MRSA identification relative to the studies that reported comparable lower prevalence (Egyir *et al*., 2014a; Egyir *et al*., 2014b; Egyir *et al*., 2016). These studies (Donkor & Nartey, 2007; Odonkor *et al*., 2012; Karikari *et al*., 2017),

together with that of Sampane-Donkor *et al.* (2017) which reported a 3.4% MRSA prevalence, identified MRSA phenotypically whereas the others, approached the identification via PCR amplification targeting the mecA gene. Yet, it cannot be emphatically asserted that the three studies in question overestimated the MRSA prevalence, as the other studies (Egyir *et al*., 2014a; Egyir *et al*., 2014b; Egyir *et al*., 2016) probably underestimated the prevalence via limiting their MRSA detection strategy to mecA gene detection. This may be so, as the mecA homologue, mecC, also modulates methicillin resistance (Cartwright *et al*., 2013; Petersen *et al*., 2013). Moreover, there may be other molecular determinants of methicillin resistance which are yet to be discovered. Consequently, exploring the molecular determinants of methicillin resistance presents a study area that warrants continuous attention from researchers. That said, in estimating MRSA prevalence, phenotypic detection needs to be a pre-requisite, with the molecular detection serving as an adjunct.

2.4 Sickle cell disease (SCD) and MRSA

"Sickle cell disease is a collective term for the description of genetic disorders that eventually culminate in an episodic formation of sickle-shaped red blood cells (RBCs)". "It affects millions of people worldwide (Modell *et al*., 2008), particularly those of black African (Herrick *et al*., 2014), AfroCaribbean, Mediterranean, and Middle East descent (Dick *et al*., 2007)". "About 75% of people with HbSS disease are in sub-Saharan Africa (WHO, 2010)". "A single nucleotide substitution of the beta-globin coding gene on chromosome 11, which leads to the replacement of glutamic acid residue with valine on the surface of the protein is the root cause of the resultant mutant usually termed HbS". "Severe form of this condition is observed in homozygous SS individuals. This is characterized by predisposition to an array of interrelated

clinical conditions, crisis and infections such as osteomyelitis (Chambers et al., 2000), vaso-occlusion (Madigan et al., 2006), hemolysis which leads to chronic anaemia (Suter et al., 2003), as well as acute splenic sequestration (Khatibet al., 2008)".

However, infections, and not crisis, is the prominent factor which precipitates the risk of SCD-associated morbidity and mortality, particularly in children. Bacterial infections have over the years dominated as the common cause of hospitalization in SCD children (Aken'Ova *et al*., 1998). One major clinical intervention in the quest to increase life expectancy in SCD infants and children has been the routine use of pneumococcal prophylaxis in developed countries (John *et al*., 1984; Gaston *et al*., 1986), which has lessened the burden of invasive pneumococcal diseases and increased survival (Lee *et al*., 1995). A similar story cannot be told for *S. aureus*, although it has also been implicated in most invasive diseases among HbSS people (Okuonghae *et al*., 1993) chaperoned by diverse resistant mechanisms such as in MRSA strains.

Sickle cell disease constitutes an unrecognized risk group for *S. aureus* and MRSA carriage. This may explain why there is a paucity of information on carriage of *S. aureus* and MRSA in individuals with sickle cell disease. An extensive literature search revealed only two published studies on the subject – that of Donkor *et al*. (2013) conducted in Ghana and that of Schaumburg *et al*. (2013).

"In the study of Donkor *et al*. (2013), the researchers explored the possible predisposition of individuals with sickle cell disease to harbor antibiotic resistant *Staphylococcus aureus* and *Streptococcus pneumoniae"*. "The researchers prospectively sampled 50 children each of HbSS and non-HbSS genotypes in a cross-sectional study carried out between 2006 and 2007". "The carriage prevalence of *S. aureus* in the children with HbSS and non-HbSS genotypes respectively were found to be 48% and 50% respectively, and this difference in prevalence was not

statistically significant". "The rates of antibiotic resistance for the *S. aureus* isolates, for children with HbSS and non-HbSS genotypes respectively were penicillin (100% vs. 88%), ampicillin (100% vs. 88%), cefuroxime (75% vs. 58%), erythromycin (33% vs. 13%), cloxacillin (6% vs. 0%), and co-trimoxazole (56% vs. 79%)".

In the study conducted in 2010 by Schaumburg *et al*. (2013) among 73 sickle cell disease children and 143 children without the disease (control group), which was similar in design to the study by Donkor *et al*. (2013), the researchers reported carriage prevalence of 46.6% and 46.9% respectively in the sickle cell disease and control participants respectively. The rates of antibiotic resistance for the *S. aureus* isolates, for sickle cell disease children and control participants respectively, were penicillin (100% vs. 97.2), ampicillin (100% vs. 97.2), oxacillin (2.2% vs. 1.4%), clindamycin (8.7% vs. 1.4%), macrolides (13.0% vs. 5.6%), tetracycline (54.3% vs. 50%), and co-trimoxazole (45.7% vs. 43.1%).

Given the limited number of *S. aureus* carriage studies among individuals with sickle cell disease, the subject matter of *S. aureus* carriage in these individuals could rightly be viewed as a "green area", and hence meriting attention from researchers.

"CHAPTER THREE"

3.0 "METHODOLOGY"

3.1 Study area

"This study was conducted at the sickle cell clinic of the Princess Marie Louise Children's Hospital in Accra, Ghana. According to the Ghana Statistical Service (2012), the city is inhabited by about two million people, and has 27 hospitals. PML is the only hospital in Ghana that primarily specializes in pediatric care". Its sickle cell clinic attends to sickle cell disease patients once a week – Thursdays. On the average, annually, 414 children attend the Sickle cell clinic with an arranged 3 months period for the next visit (personal communication).

3.2 "Study design and sampling"

"The study was cross-sectional, and involved 120 children with sickle cell disease (both HbSS and HbSC genotypes) and 100 children without the disease (control group) recruited between March and August, 2018". The HbSS children were recruited from the Sickle Cell Clinic of the hospital, and the control group was recruited from the environs of the hospital. The inclusion criteria for selection of the participants constituting the sickle cell disease group were: being a child with laboratory-proven HbSS or HbSC genotype in a steady state, being between 1 and 13 years of age, and being an outpatient. In the selection of the participants of the control group, the inclusion criteria satisfied were: being a child with laboratory-proven HbAA genotype, being apparently healthy, and being between 1 and 13 years of age. In both study groups, the exclusion criteria were: being on antimicrobials (other than penicillin) two weeks prior to sampling, having

known co-morbidities, and inability to determine the haemoglobin genotype of potential participants.

The minimum sample size was determined as follows:

$$n = \frac{z^2 \times p\,(1-p)}{m^2}$$

Description: n= minimum sample size

z = confidence level at 95% (standard value of 1.96)

m = margin of error at 5% (standard value of 0.05)

p = estimated MRSA nasopharyngeal carriage prevalence = 3.4% or 0.034 (Sampane-Donkor *et al*., 2017).

$$n = \frac{1.96^2 \times 0.034\,(1-0.034)}{(0.05)^2} = 50.57$$

Thus the minimum sample size was calculated to be 51. In order to further increase the statistical power of the study, the sample size was increased to 120 for the sickle cell disease participants and 100 for the control group.

3.3 Specimen and risk factors data collection

Swabs were obtained from the anterior nares of the study participants between March and August, 2018. Collection of the swab specimens were done by a qualified paediatrician after obtaining informed consent from the guardians of the study participants. For each participant, a

sterile cotton swab was rotated five times in both anterior nares. The swab specimen was then placed in a pre-labeled vial containing 1ml skim milk-tryptone-glucose-glycerin (STGG) medium and cut aseptically with a pair of scissors (which had been disinfected using a 70% alcohol wipe) such that the swab and a part of its handle, totalling about 4cm in length, would remain in the medium-contained vial. The cap of the vial was then tightened, and the specimen was maintained on ice, and transported within four hours to the research laboratory of the Department of Medical Microbiology (SBAHS). The specimens were then vortexed for about two minutes and stored in a -80 centigrade freezer until needed.

Furthermore, a pretested structured questionnaire (Appendix III) was used to collect data on risk factors for *S. aureus* carriage from the study participants. The questionnaire was divided into three parts, namely, demography, household characteristics, and clinical features, and the responses were obtained by interview and review of patients' folders.

3.4 Analysis of specimen

The specimens were pre-enriched in 5ml of tryptic soy broth and incubated at 37ºC for 24 hours. Each 24-hour culture was inoculated onto blood, chocolate, and MacConkey agars, with a sterile inoculation loop. The plates were incubated at 37ºC in 5% CO2, with the exception of the MacConkey's agar plates which were incubated aerobically. After 24 hours of incubation, the plates were examined for growth, and follow up was done on presumptive staphylococcal colonies.

3.5 Identification of *Staphylococcus aureus* and antimicrobial susceptibility testing

The staphylococcal isolates were classified as *S. aureus* or coagulase negative staphylococci based on their colonial morphology and their reaction to Gram stain, catalase test, and coagulase test. The isolates that were coagulase positive were identified as *S. aureus,* and those that were coagulase negative were identified as coagulase negative staphylococci. Antimicrobial susceptibility patterns of all the *S. aureus* isolates was determined by the Kirby-Bauer disc diffusion method, following the Clinical Laboratory Standards Institute (CLSI, 2017) recommendations for Antimicrobials Susceptibility Testing (AST). The antibiotics used included: gentamicin (10µg), rifampin (5µg), linezolid (10µg), fusidic acid (10µg), cefoxitin (30µg), ciprofloxacin (10µg), tetracycline (30µg), clindamycin (2 µg), erythromycin (15 µg), penicillin (1unit), and sulfamethoxazole trimethoprim (1.25+23.75 µg). Isolates that displayed resistance to cefoxitin (breakpoint less than or equals to 21mm) were identified as MRSA. *S. aureus* strains American Typed Collection Culture (ATCC) 43300 and ATCC 25923 were used as quality control strains for the AST. Results were interpreted according to the performance standards of CLSI (2017) guidelines on AST.

3.6 Molecular investigations

DNA was extracted from the MRSA isolates, and screened for the presence of the of the *mec*A gene using Polymerase Chain Reaction (PCR) as described by Larsen et al, (2008). Extraction of genomic DNA was done from overnight Lysogeny broth (LB) cultures of the seven cefoxitin-resistant *S. aureus* isolates (for both the HbSS-children and HBAA-participants) and a positive control isolate using the Zymo Research extraction kit, following the instructions of the

manufacturer. For quality control purposes, 5 µL of the extracted DNA from each isolate was mixed with 2 µL of bromophenol blue gel loading buffer and ran on a 1.2% agarose gel, and the bands visualized by UV illumination. Carriage of the *mec*A and *nuc*A genes were detected by conventional PCR amplification using the extracted DNA samples as templates. The negative control used was RNase-free water. The total reaction volume for the PCR was 50 uL, consisting of the genomic DNA (of final concentration 60 ng/µL), PCR water, primers (of final concentration 0.2 µM), Taq polymerase (of final concentration 1.25 U/µL), $MgCl_2$ (of final concentration 2 mM), and dNTPs (of final concentration 200 µM). The amplicons were visualized by UV illumination, preceded by separation by a 1.2% agarose gel electrophoresis.

For the *mec*A PCR, the primer sequences used were ATCGATGGTAAAGGTTGGC (forward primer) and AGTTCTGCAGTACCGGATTTGC (reverse primer) (Sajith Khan *et al*., 2012), and the cycling conditions were: initial denaturation at 95°C for 3 minutes; denaturation at 94°C for 1 minute; annealing at 55°C for 30 seconds; extension at 72°C for 1 minute; final extension at 72°C for 6 minutes; the total number of cycles was 33.

Using the same cycling conditions as those used in the *mec*A PCR, a *nuc*A PCR was performed on all the DNA samples, including those of cefoxitin-resistant *S. aureus* descent that showed no bands for the *mec*A genes, in order to ascertain that *S. aureus* ancestors of those DNA samples were in fact, *S. aureus* (Brakstad *et al*., 1992). The primer sequences used were GCGATTGATGGTGATACGGTT (forward primer) and AGCCAAGCCTTGACGAACTAAAGC (reverse primer) (Brakstad *et al*., 1992).

3.7 Ethical considerations

All procedures undertaken in this study were done in consonance with ethical guidelines, including demonstrating a high measure of respect for the rights and confidentiality of all the study participants. Approval for the conduction of this study was given by the Ethical and Protocol Review Committee of the College of Health Sciences. Furthermore, parents/guardians of the children gave their informed consent before the children were recruited as study participants, and children between six and fourteen years of age were required to give their assent. In addition, the risks and benefits associated with the study were clearly explained to the prospective study participants in order for them to make an informed choice of enrolling in the study. All data, both electronic and physical, were stored in secure password-protected archival systems.

3.8 Data Analysis

Data were analyzed with the help of Stata, version 14. "Data on resistance of *S. aureus* to the antimicrobials tested were summarized using descriptive statistics". Independent sample Chi-square tests were performed to determine the association between children with HbSS and *S. aureus* and MRSA colonization, and also, within each group, association between individual categorical risk factors and *S. aureus* and MRSA colonization. "A point biserial correlation was performed to determine association between risk factors that were continuous variables and *S. aureus* and MRSA colonization". Finally, risk factors that showed significant association with colonization in the Chi-square and point biserial correlation tests were put in a binary "logistic regression model to determine their predictive value of colonization".

"The significance of each predictor variable of colonization was assessed by determining the *p* value, odds ratio, and confidence interval; *p* values less than 0.05 were considered significant".

CHAPTER FOUR

4.0 RESULTS

4.1 "Demographic, household, and clinical characteristics of the study participants"

In total, two hundred and twenty (220) individuals participated in this study. Of this number, the sickle cell disease (SCD) children comprised one hundred and twenty (120), whereas the participants of the control group comprised hundred (100). "The mean age and BMI of the sickle cell disease children were 5.84 years and 15.37 Kg/m^2 respectively, and the corresponding values within the control group were 6.36 years and 19.80 Kg/m^2 respectively". In the sickle cell group, the gender distribution was uneven (48.3% males vs. 51.7% females), but was even in the control group (the males and females comprised 50% each of the population). In both study groups, majority of the participants were enrolled in school (86.7% in the SCD group vs. 97% in the control group), resided in compound houses (69.2% in the SCD group vs. 61% in the control group), and often washed their hands with soap (58.3% in the SCD group vs. 55.0% in the control group), and at least, 97% of the participants had no health worker present in their household. Table 1 presents the demographic and household characteristics of the study participants.

"With regard to the clinical features of the study participants, they are presented in Table 2". As evident in the table, the sickle cell disease children were predominantly (96.7%) on penicillin prophylaxis, whereas that attribute was absent among the participants of the control group. Also, relatively few of the study participants reported that they practiced self-medication (20% in the SCD group vs. 23% in the control group), had a history of hospitalization in the past year (40.8%

in the SCD group vs. 8.0% in the control group), or a history of pneumonia (6.7% in the SCD group vs. 0.0% in the control group).

"Table 1: Demographic and household characteristics of the study participants"

Demographic and household characteristics		SCD Children Mean age- 5.84		Control Mean age- 6.36	
		Number	**%**	**Number**	**%**
Age	1-5	68	56.7	41	41
	6-13	44	43.3	59	59
Gender					
	Male	58	48.3	50	50.0
	Female	62	51.7	50	50.0
Current school enrolment					
	Yes	104	86.7	97	97.0
	No	16	13.3	3	3.0
Type of residence					
	Self-contained	37	30.8	38	38.0
	Compound	83	69.2	61	61.0
Presence of health worker in household					
	Yes	3	2.5	0	0
	No	117	97.5	100	100
Hand washing with soap					
	Rarely	50	41.7	45	45.0
	Often	70	58.3	55	55.0

Age (SCD children = 5.84 ± 2.99 years; Control = 6.36 ± 3.45); BMI (SCD children = 15.37 ± 10.91 Kg/m^2; Control = 19.80 ± 9.45 Kg/m^2); Number of individuals per household (SCD children = 8.14 ± 3.32; Control = 7.14 ± 2.80)

"Table 2: Clinical features of the study participants"

Clinical features	SCD Children		Control	
	Number	%	Number	%
Self-reported self-medication				
Yes	24	20	23	23.0
No	96	80	77	77.0
Penicillin prophylaxis				
Yes	116	96.7	0	0
No	4	3.3	100	100
History of hospitalization in the past year				
Yes	49	40.8	8	8.0
No	71	59.2	92	92.0
Chronic skin condition				
Yes	0	0	0	0
No	120	100	100	100
History of pneumonia				
Yes	8	6.7	0	0
No	112	93.3	100	100
History of TB				
Yes	0	0	0	0
No	120	100	100	100
History of surgery				
Yes	0	0	0	0
No	120	100	100	100
Underlying disease				
Yes	0	0	0	0
No	120	100	100	100
History of blood transfusion				
Yes	42	35	0	0
No	78	65	100	100

Frequency of hospitalization per year (SCD children = 0.70 ± 1.37 times; Control = 0.10 ± 0.41)

4.2 "Relationship between sickle cell disease and staphylococcal carriage"

As observed in Table 3 below, the distribution of the Staphylococci isolated from the study participants were as follows *Staphylococcus aureus* (33.3% in the sickle cell disease children vs. 10% in the control participants), coagulase-negative staphylococci (7.5% in the sickle cell disease children vs. 8.0% in the control participants), and MRSA (9.2% in the sickle cell disease children vs. 5.0% in the control participants). Considering both study groups as a composite, the overall nasal carriage prevalence of *S. aureus*, MRSA, and coagulase-negative staphylococci were 22.73%, 7.27, and 7.73%.

"Moreover, a significant association was observed between presence of sickle cell disease and *S. aureus* carriage [$\chi^2 = 16.91$, $p < .0001$], but this was not observed in relation to MRSA carriage [$\chi^2 = 1.4$, $p = .237$]". The odds ratio of S. *aureus* carriage in relation to the presence of sickle cell disease was 4.045. This means that sickle cell disease children have more than a four-fold increased risk for *S. aureus* carriage.

Table 3: Staphylococci isolated from the study participants

Staphylococci isolated	SCD children		Control	
	Number	Prevalence (%)	Number	Prevalence (%)
Staphylococcus aureus	40	33.3	10	10.0
MRSA	11	9.2	5	5.0
CONS	9	7.5	8	8.0

As observed in Figure 1 below, presence of the *mec*A gene was demonstrated in only four of the total sixteen MRSA isolates, and these originated from participants of the sickle cell group. Subsequent PCR targeting the *nuc*A gene confirmed these and all but two of the isolates that showed negative results for *mec*A gene presence to be *S. aureus*, and this is demonstrated in Figure 2 below.

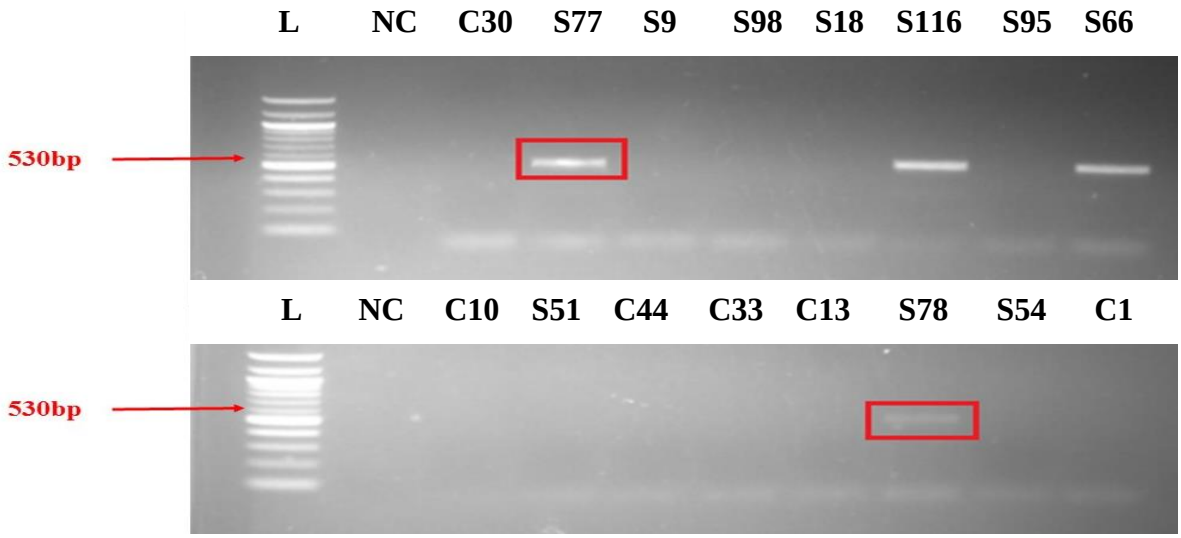

Figure 1: Results for *mec*A gene screening

In the figure, L represents Ladder; NC represents negative control; numbers preceded by "C" represent *S. aureus* isolated from participants of the control group; numbers preceded by "S" represent *S. aureus* isolated from participants of the sickle cell disease group

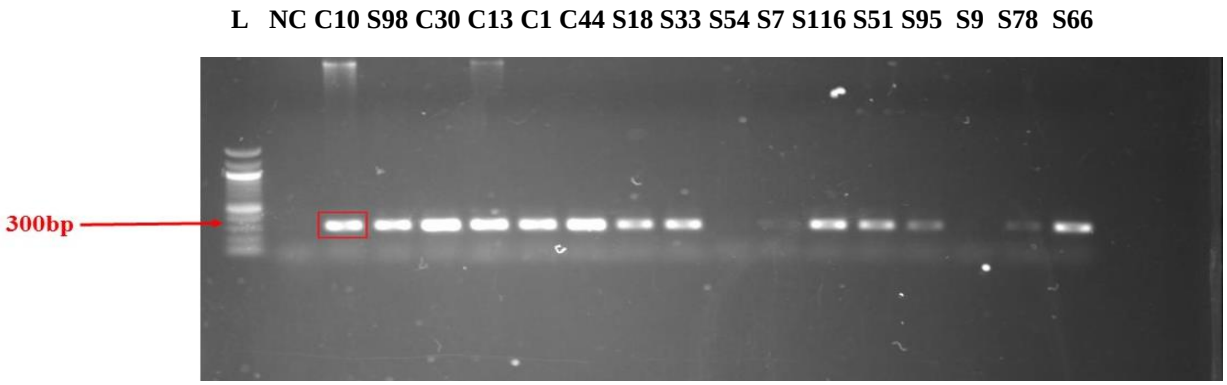

Figure 2: Results for *nuc*A gene screening

In the figure, L represents Ladder; NC represents negative control; numbers preceded by "C" represent *S. aureus* isolated from participants of the control group; numbers preceded by "S" represent *S. aureus* isolated from participants of the sickle cell disease group

4.3 "Risk factors for colonization with *S. aureus* and MRSA among the study participants"

"The results of the logistic regression analysis indicated that increasing age, male gender, living in self-contained apartments, and practice of self-medication were risk factors for carriage of *S. aureus* among the sickle cell disease children". "However, none of these and the other factors assessed were determinants of MRSA carriage". "Moreover, history of hospitalization in the past year was a risk factor for both *S. aureus* and MRSA carriage in the control group". Details of the risk factor analysis are presented in Table 4.

Table 4: Risk factors for *S. aureus* and MRSA colonization

	SCD children		Control	
Risk factor	**OR (95% CI)**	***p* value**	**OR (95% CI)**	***p* value**
Age*	1.275 (1.084–1.499)	.003	N/A	N/A
Male gender*	0.344 (.142–.833)	.018	N/A	N/A
Living in SC apartments*	3.632 (1.108–11.906)	.033	N/A	N/A
Practice of self-medication*	.233 (.059–.927)	.039	N/A	N/A
Hospitalization in the past year*	N/A	N/A	14.333 (1.023–200.907)	0.048
Hospitalization in the past year+	N/A	N/A	21.176 (1.084–413.823)	0.044

*associated with *S. aureus* colonization; +associated with MRSA colonization; N/A = Not applicable; SC = Self-contained

4.4 "Patterns of antimicrobial resistance among the *S. aureus* and MRSA isolates"

In both study groups, all the *S. aureus* isolates were penicillin-resistant. Also, relatively higher rates of resistance was also observed for fusidic acid (95% in the SCD children vs. 80% in the control group) and rifampicin (82.5% in the SCD children vs. 50% in the control group), which was the only antibiotic whose differences in resistance rates reached statistical significance ($z = 2.152$; $p = 0.03$). The rates recorded against the other antimicrobials were: gentamicin (7.5% in the SCD children vs. 20% in the control group), clindamycin (32.5% in the SCD children vs. 50% in the control group), erythromycin (30% in the SCD children vs. 20% in the control group), linezolid (30% in the SCD children vs. 40% in the control group), tetracycline (25% in the SCD children vs. 50% in the control group), and co-trimoxazole (27.5% in the SCD children vs. 20% in the control group). The rates of multidrug resistance were 92.5% in the SCD group ($n = 37$) and 100% ($n = 10$) in the control group. In Figure 3, the rates of antimicrobial resistance are presented, and in Table 5, the antibiogram of the MRSA isolates are presented.

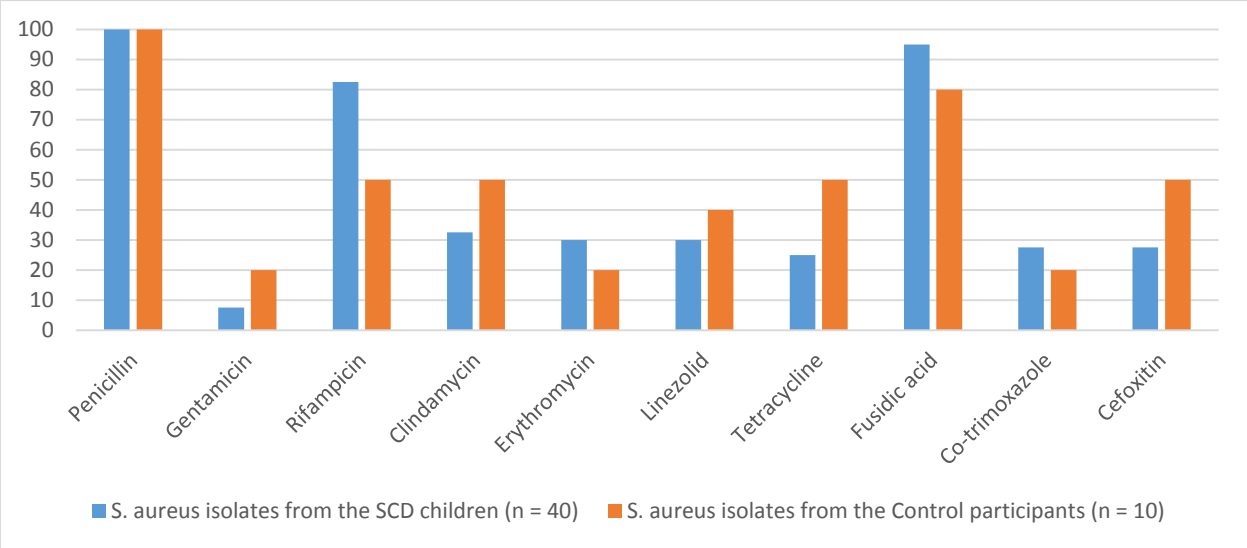

***Figure* 3: "Antibiotic resistance rates of the *S. aureus* isolates"**

Table 5: Antimicrobial resistance patterns of the MRSA isolates

"Isolates /	C	S	C	C	C	C	S	S	S	S	S	S	S	S	S	S
Antimicrobials	**10**	**98**	**30**	**13**	**1**	**44**	**18**	**33**	**54**	**77**	**116**	**51**	**95**	**9**	**78**	**66**
Tetracycline	S	S	R	R	S	R	IR	S	R	R	S	R	IR	S	R	S
Erythromycin	IR	S	IR	S	S	IR	IR	IR	IR	S	S	R	S	R	R	S
Gentamicin	R	S	S	S	S	R	S	S	S	S	S	S	S	R	IR	S
Rifampicin	IR	R	IR	R	R	IR	R	R	R	R	S	R	R	R	R	R
Cotrimoxazole	S	S	S	R	R	S	S	IR	IR	R	S	R	IR	S	R	IR
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Clindamycin	IR	IR	R	S	IR	R	IR	R	IR	S	S	IR	R	IR	IR	R
Fusidic acid	R	R	R	S	R	R	R	R	R	R	S	R	R	R	R	R
Cefoxitin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Linezolid	S	S	S	S	S	R	S	R	S	S	S	R	S	S	S	R"

"S = Sensitive; R = Resistant; IR = Intermediate resistant;"

Numbers preceded by "C" and "S" represent isolates emanating from control and SCD children respectively

CHAPTER FIVE

5.0 DISCUSSION

5.1 Staphylococci carried by the study participants

One of the objectives of this study was to determine the distribution of Staphylococci colonizing the anterior nares of the study participants. Whether each of the study groups are considered exclusively, or as a composite, *S. aureus* was the predominant colonizer. The overall nasal MRSA carriage was 7.27%, being comparable to CONS carriage – 7.73%.

The predominance of *S. aureus* in the anterior nares of participants of both study groups may be as a consequence of the relatively lower carriage of coagulase negative staphylococci in both study groups. In fact, data from previous studies suggest coagulase-negative staphylococcal carriage to be antagonistic to *S. aureus* carriage (Iwase *et al*., 2010; Olson *et al*., 2014; Paharik *et al*., 2017; Donkor *et al*., 2019). Moreover, in another study involving children (Donkor & Nartey, 2007), a similar carriage prevalence of *S. aureus* relative to CONS was reported – 49% vs. 27.4%.

"The carriage prevalence of MRSA in this study is high when placed in the context of what has been reported in other recent carriage studies among children in the country (< 4%) (Eibach *et al*., 2017; Donkor *et al*., 2019)". "Although this may represent an isolated scenario, it may also be an indicator of a gradual spread of the pathogen or an impending outbreak, and hence may require public health attention". Moreover, their occurrence in these children, especially, those with SCD is cause for worry, given the impairment of their immune systems.

5.2 Relationship between sickle cell disease and *S. aureus* and MRSA carriage

"Another aspect of this study was to determine the relationship between the presence of sickle cell disease and *S. aureus* and MRSA nasal carriage". This study seems to be one of the few to evaluate carriage of these organisms among "children with sickle cell disease" in relation to those without the disease. Overall, the presence of sickle cell disease predisposed children to "nasal carriage of *S. aureus*, but not MRSA".

In a previous study that partly focused on comparing cohorts of children with and without SCD in the country on their predisposition to *S. aureus* carriage (Donkor *et al*., 2013), the *S. aureus* carriage prevalence was not significantly different. In fact, the carriage prevalence was higher in the control group (48% vs. 50%). This contrasts the 33.3% and 7.5% *S. aureus* nasal carriage prevalence respectively recorded in the SCD children and the control participants in the current study. Another contrasting observation to that of this study is seen in one similar study conducted in Gabon (Schaumburg *et al*., 2013), which reported a finding similar to that of Donkor *et al*. (2013) – 46.6% of the sickle cell disease children were *S. aureus* carriers as opposed to 46.9% in the control group.

Consequently, this study contributes to identify people with sickle cell disease as a risk group for *S. aureus* carriage. Although additional studies could be conducted to ascertain this finding, this observation probably explains the high occurrence of invasive diseases caused by *S. aureus* in sickle cell disease patients (Okuonghae *et al*., 1993; Akuse *et al*., 1996; Aken'Ova *et al*., 1998; Thanni *et al*., 2006; Kizito *et al*., 2007).

The identification of individuals with sickle cell disease as an at risk population for *S. aureus* carriage is additional reason for worry, given their high consumption of antibiotics, as this could make them reservoirs of multidrug resistant *S. aureus*. Interestingly, in this study, this risk group for *S. aureus* carriage were not predisposed to MRSA carriage.

It is noted that this is not the first time that *S. aureus* carriage, but not MRSA carriage, has been associated with a risk group. A previous study in the region identified HIV-infected individuals as a risk group for *S. aureus* carriage, but not MRSA carriage (Donkor *et al*., 2019). This may not necessarily be a coincidence. In a previous report by Schaumburg *et al.* (2014), immunosuppression was noted to be associated with carriage of PVL-positive *S. aureus*. As PVL characterization was not carried out in the current study and that of Donkor *et al*. (2019), an exhaustive inference cannot be drawn between the PVL status of colonizing *S aureus* strains and immunosuppression. However, as both studies were carried out in a PVL-endemic region, it is reasonable to hypothesize that PVL carriage could be a possible explanation for the linkage between immunosuppression and *S. aureus* carriage in the current study and the study conducted by Donkor *et al*. (2019). These observations reinforce the need for continued surveillance of *S. aureus* and MRSA among at risk populations.

5.3 Risk factors for colonization with *S. aureus* and MRSA

Another key objective of this study was to identify risk factors for colonization with *S. aureus* and MRSA among the study participants. Among the SCD children, none of the factors studied were predictive of MRSA carriage. However, increasing age, male gender, living in self-contained apartments, and practice of self-medication were identified as risk factors for *S. aureus*

nasal carriage. In the control group, history of hospitalization in the past year was a risk factor for both *S. aureus* and MRSA nasal carriage. Da Silveira et al., (2018), identified living in small and medium sized facilities as well as recent hospitalization as risk factors for MRSA colonization among elderly living in nursing homes. Similar to this study, recent hospitalization was the only risk factor for MRSA colonization. May be accounted for by the fact that the study participants were elderly and resident at the nursing homes compared our participants who were outpatients.

Age has been noted to have an inverse relationship with *S. aureus* carriage (Williams, 1963; Olsen *et al*., 2009). Hence the contrasting finding that increasing age is a risk factor for *S. aureus* carriage is difficult to explain. It may be a chance finding, or could probably be attributed to differences in population dynamics, and there may be a need to rely on future research to improve insights on the observation.

Some studies have failed to demonstrate any significant association between gender and *S. aureus* carriage (Egyir *et al*., 2014; Ateba *et al*., 2012; Ebruke *et al*., 2016). Be that as it may, male gender has been associated with *S. aureus* carriage in other studies (Bogaert *et al*., 2004; Kuehnert *et al*., 2006; Seybold *et al*., 2009; Olsen *et al*., 2012; Lewnard *et al*., 2016). The reason for male gender emerging as a risk factor could be as a result of their deficiency in estrogen relative to female gender. Female gender, by virtue of estrogen production, has been linked with better immune function (Beery, 2003; Marriott & Huet-Hudson, 2006).

Similar to the phenomenon with age in the current study, it is difficult to pinpoint why living in self-contained apartments would predispose to *S. aureus* carriage, as was observed among the

SCD children in this study, even though attempts could be made to explain the reason for the occurrence. One possible explanation for the phenomenon could be that the *S. aureus* carriers among the SCD children may have acquired their carrier states from colonized household members. Past studies have demonstrated the importance of close contacts within households and with parents in spread of *S. aureus* carriage among children residing in the same household (Miller *et al*., 2009; Regev-Yochay *et al*., 2009). Nonetheless, as this study was not designed to include an evaluation of *S. aureus* carriage in household members of the study participants, these attempts at explanations are at best, educated guesses.

In addition to age, male gender, and living in self-contained apartments, self-medication emerged as significant risk factor for *S. aureus* colonisation among the HIV infected children. Even though a previous study by Lemma *et al*. (2015) reported history of antibiotic use over the previous 3 months to be a significant risk factor for *S. aureus* colonisation among children aged between 7 and 15 years, the researchers failed to indicate whether those individuals with history of antibiotic usage administered the drugs by themselves (self-medication) or with doctors' prescription. Hence, it does not seem legitimate to compare findings of this current study with that reported by Lemma *et al*. (2015). Moreover, even though antibiotic use has been reported to predispose to MRSA carriage too (Fritz *et al*., 2008), it is noted that this study evaluated whether or not the study participants practiced self-medication, but did not evaluate the drugs used in the practice. Moreover, the variable "self-medication" is limited by the fact that it only encompasses what was reported by the study participants, and hence may not reflect self-medication in the absolute sense. Hence a measure of caution is warranted in the interpretation of this finding. Regardless of the aforementioned technicalities, this finding underpins the need to evaluate self-

medication practices in an exhaustive manner when carrying out *S. aureus* and MRSA surveillance studies, as well as the need to focus more studies on self-medication.

Also, it necessitates the need for improved campaigns against self-medication, particularly, among sickle cell disease children. This is important, given the extensive marketing of drugs in the country, coupled with their ready availability (Lee *et al*., 1991; Adu-Sarkodie, 1997; Reeves, 2007)

Finally, the observed predisposition of individuals with history of hospitalization in the control group to *S. aureus* and MRSA nasal carriage was not surprising. Yet, it is important to highlight that it has not been universally established as a risk factor. To illustrate, although some studies have failed at demonstrating previous hospitalization as a risk factor for *S. aureus* carriage (Seybold *et al*., 2009; Lemma *et al*., 2015), while others have demonstrated that it predisposes to MRSA carriage (Madani *et al*., 2001; Hidron *et al*., 2005; Köck *et al.*, 2014). Thus this study seems to be the first to report history of hospitalization as a risk factor for both *S. aureus* and MRSA colonization. It additionally provides insights into the predisposition of individuals whose household members are employed in healthcare to *S. aureus* and MRSA carriage (Creech *et al*., 2005; Fritz *et al*., 2008).

5.4 Patterns of antimicrobial resistance among the *S. aureus* and MRSA isolates

A final objective of this study was to determine the patterns of antimicrobial resistance among the *S. aureus* and MRSA isolates. In both study groups, all the *S. aureus* isolates were penicillin-resistant, and majority of them, at least, 80%, displayed resistance towards fusidic acid. The high rates of resistance recorded against penicillin was expected, given the high rates of resistance (> 80%) recorded against the antibiotic by *S. aureus* in several studies (Donkor *et al*., 2013; Egyir *et*

al., 2013; Egyir *et al*., 2014; Dekker *et al*., 2016; Eyir *et al*., 2016). These studies additionally reported *S. aureus* resistance to fusidic acid at rates of 0–12%, and hence the high rates of fusidic acid resistance observed in this study could either be an isolated case or could predict the beginning of an obsolete-rendering of fusidic acid as a therapeutic agent against *S. aureus* infections. For erythromycin, linezolid, and co-trimoxazole, the rates of resistance against them were similar in both groups, and generally ranged between 20% and 40%. Earlier studies conducted within the country (Donkor *et al*., 2013; Egyir *et al.*, 2013; Egyir *et al*., 2014; Dekker *et al*., 2016; Eyir *et al*., 2016) have reported comparable rates for erythromycin, but higher rates for co-trimoxazole. With regard to linezolid resistance, although the rates of 30% and 20% respectively reported in the SCD and control groups in this study might seem moderate, they are cause for worry. This is because generally, *S. aureus* resistance to linezolid is rare (Howe *et al*., 2002; Morales *et al*., 2010; Long & Vester, 2012; Shore *et al*., 2016), and the situation is no different in Ghana (Egyir *et al*., 2013; Donkor *et al*., 2019). Also, the antibiotic is one of the few therapeutic options for treating MRSA infections. Besides, it has a low coverage in the country. Evidently then, it may be necessary to design studies to screen for the plasmid-borne *cfr* gene as well as other linezolid resistance determinants among pathogens in the country. Although there seemed to be disparities between the two study groups with regard to the rates of resistance of the *S. aureus* isolates to gentamicin, clindamycin, and tetracycline, the apparent disparities constituted a chance finding. Nonetheless, the rates of resistance are consistent with what have been reported in previous studies (Dekker *et al*., 2016; Sampane-Donkor *et al*., 2017; Donkor *et al*., 2019). It was resistance to rifampicin that was significantly higher in the isolates emanating from the SCD children than those of the control group. This high rate (82.5%) warrants attention, particularly, owing to the drawback it could pose to TB management should this resistance trait

be transferred to etiologic agents of TB, given that rifampicin is a backbone to TB management (Mitchison, 2000).

As the focus of this study is on sickle cell disease children, it is logical to also compare the *S. aureus* isolates obtained in this study to *S. aureus* isolates obtained from other SCD cohorts on their resistance to specific antimicrobials. Admittedly, it is the study conducted in the country by Donkor *et al*. (2013) and in Gabon by Schaumburg *et al*. (2013) that seem suitable for this comparison, given the paucity of *S. aureus* carriage studies among sickle cell disease patients. Of the antibiotics tested in this study, three were tested in the study of Donkor *et al*. (2013) – penicillin, erythromycin, and co-trimoxazole – and four were tested in the study of Schaumburg *et al*. (2013) – penicillin, clindamycin, tetracycline, and co-trimoxazole. In the study conducted by Donkor *et al*. (2013), the rates of resistance recorded against the antibiotics, by *S. aureus* isolates from sickle cell disease patients relative to control participants, were: penicillin (100% vs. 88%), erythromycin (33% vs. 13%), and cotrimoxazole (56% vs. 79%). Of these, it is the rate recorded against co-trimoxazole by isolates from SCD participants in the current study (27.5%) that seems to be a departure from the rate of 56% for the analogous isolates in the study of Donkor *et al*. (2013). As Donkor *et al*'s (2013) study was conducted between 2006 and 2007, the period that has elapsed since then (almost fifteen years), coupled with the evolutions in antimicrobial usage during the period, as well as changes in other practices which may influence antimicrobial resistance, may account for these differences. Besides, differences in population dynamics may also have played a role. Apparently, these factors have not exerted much influence on the response of *S. aureus* to penicillin and erythromycin; further research is required to substantiate this.

As regards the study conducted by Schaumburg *et al*. (2013), the rates of resistance recorded against the antibiotics, by isolates from sickle cell disease patients relative to control participants, were: penicillin (100% vs. 97.2%), clindamycin (8.7% vs. 1.4%), tetracycline (54.3% vs. 50%), and co-trimoxazole (45.7 vs. 43.1%). With the exception of the rate of resistance to penicillin which was the same as what was recorded in the current study, there were major variations between the rates of resistance observed in this study and that of Schaumburg *et al*. (2013). A major reason for this occurrence could be differences in geographical locations, as well as population dynamics.

It is noted that the phenotypic indicator, based on which methicillin resistance was evaluated, cefoxitin resistance trait, yielded results that were inconsistent with the *mec*A PCR results. Only four of the total sixteen cefoxitin-resistant *S. aureus* isolates, all emanating from the SCD participants, were confirmed to carry the *mec*A gene. Probably, those isolates in which carriage of the *mec*A gene could not be demonstrated harbored other determinants of methicillin resistance, such as the newly discovered *mec*C gene (Cartwright *et al*., 2013; Paterson *et al*., 2014).

"The rates of multidrug resistance were high in both study groups [SCD group (92.5%, n = 37); control group (100%, n = 10)]". "These rates are higher than what have been recorded in previous studies (Egyir *et al*., 2014; Dekker *et al*., 2016; Sampane-Donkor *et al*., 2017; Donkor *et al*., 2019), and may be due to extensive marketing of antimicrobials and indiscriminate antibiotic use (Lee *et al*., 1991; Adu-Sarkodie, 1997; Donkor *et al*., 2012a)". Evidently, policy makers and implementers would have to step up public health campaigns against antimicrobial abuse and institute more stringent policies on antimicrobial acquisition.

"CHAPTER SIX"

"6.0 CONCLUSIONS, RECOMMENDATIONS, AND LIMITATIONS"

6.1 Conclusions

The main conclusions of the study are as follows:

Among the sickle cell disease children, the distribution of Staphylococci was as follows: *Staphylococcus aureus* (33.3%), MRSA (9.2%), and coagulase-negative staphylococci (7.5%). The distribution in the control group was as follows: *Staphylococcus aureus* (10%), MRSA (5.0%), and coagulase-negative staphylococci (8.0%).

Also, among children in Accra, sickle cell disease is a predisposing factor to carriage of *S. aureus*, but not MRSA; the odds ratio of S. *aureus* carriage in relation to the presence of sickle cell disease was 4.045.

In addition, increasing age, male gender, living in self-contained apartments, and practice of self-medication were risk factors for carriage of *S. aureus* among the sickle cell disease children. "The only risk factor for MRSA carriage, observed only in the control group, was history of hospitalization in the past year, and it doubled as the risk factor for *S. aureus* carriage".

Finally, both the sickle cell disease children and participants of the control group harbored multidrug resistant *S. aureus*, and this may be due to extensive antimicrobial use in the country.

6.2 “Recommendations”

“Based on the findings in this study, the following are recommended:”

“Additional studies need to be conducted to ascertain whether sickle cell disease constitutes an independent risk factor for *S. aureus* carriage, and these studies could be designed to be longitudinal”. “Also, there is a need for continued surveillance of *S. aureus* and MRSA among at risk populations”. Moreover, policy makers and implementers need to step up public health campaigns against antimicrobial abuse and institute more stringent policies on antimicrobial acquisition.

6.3 Limitations

The limitations of the study include the following:

“As this was a cross-sectional study, it precludes the making of distinctions between persistent and intermittent carriers; some intermittent carriers may have been non-carriers at the time of sampling”.

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APPENDIX I

RESEARCH CONSENT FORM

Investigator: Miss Vera Ama Appiah

Institution: Department of Medical Laboratory Sciences, SBAHS.

Research title: Nasal colonization with methicillin-resistant *Staphylococcus aureus* among sickle cell disease children

Invitation to participate in the research:

Staphylococcus aureus is a bacterium that is found in the nose and skin. It can spread to other parts of the body from these sites and cause a wide range of infections in their hosts, and can also spread to others. Some strains of this bacterium cannot be treated with methicillin because they have acquired resistance to that antimicrobial. Those strains are called methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA strains cause a wide range of serious diseases in people, and it is possible that sickle cell disease patients are at risk of its infections. Also, infections caused by MRSA are difficult to treat. It is therefore important to investigate nasal colonization of MRSA in sickle cell disease children, in order to contribute information necessary in the management of these patients to promote their health.

The collection process will involve a routine clinical procedure by qualified personnel.

Duration of the study: Anterior nasal swabs will be collected within a three-month period.

Benefits of the study:

MRSA nasal colonization studies will provide a baseline data on MRSA prevalence and antimicrobial resistance among sickle cell disease patients. This information is necessary for effective management of sickle cell disease children.

Use of collected material: Bacteria will be isolated from the samples collected from all the study participants. These samples will be destroyed by burning after 2 years. The bacteria may be subjected to genetic studies, and may be preserved in the freezer for future investigations.

Potential hazards of the study:

There may be a slight discomfort when taking the anterior nasal swab specimens from your ward.

Participant's rights to refuse or withdraw:

If you have read this form, or the content herein has been clearly explained to you, and you have agreed to let your ward participate in this study, please note that participation is voluntary and you have the right to withdraw your consent or discontinue participation in the project at any time without penalty. It is also your right to refuse to answer questions you are not comfortable with.

Confidentiality:

All collected materials from your ward will be coded using numbers and letters. The privacy of your ward will be maintained in all published and written data resulting from the study.

Questions, concerns, or complaints:

If you have any issues relating to this study, please direct them to Prof. Eric Sampane-Donkor (0553527140), Dr. George Antepem Pesewu (0277301300), or Miss Vera Appiah (0503999258), of the Department of Medical Microbiology, SBAHS.

Consent for inclusion: If you agree to the inclusion of your ward in this study, please complete the form below;

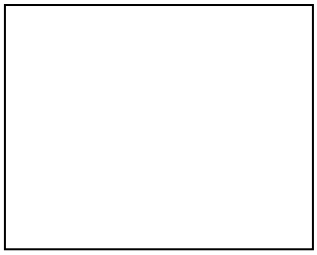
I .....…… on this day …….. (Day/Month/Year) attest that I understand the explanations given in the

consent form and thus give permission to Miss Vera Appiah to include my ward in the research study titled "Nasal colonization with methicillin-resistant *Staphylococcus aureus* among sickle cell disease children".

Signature of participant's guardian: …….....

Contact address: .....……..

Phone number: …….....



Thumb print (where required)

APPENDIX II

RESEARCH ASSENT FORM

Investigator: Miss Vera Ama Appiah

Institution: Department of Medical Laboratory Sciences, SBAHS.

Research title: Nasal colonization with methicillin-resistant *Staphylococcus aureus* among sickle cell disease children

Invitation to participate in the research:

Staphylococcus aureus is a bacterium that is found in the nose and skin. It can spread to other parts of the body from these sites and cause a wide range of infections in their hosts, and can also spread to others. Some strains of this bacterium cannot be treated with methicillin because they have acquired resistance to that antimicrobial. Those strains are called methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA strains cause a wide range of serious diseases in people, and it is possible that sickle cell disease patients are at risk of its infections. Also, infections caused by MRSA are difficult to treat. It is therefore important to investigate nasal colonization of MRSA in sickle cell disease children, in order to contribute information necessary in the management of these patients to promote their health.

The collection process will involve a routine clinical procedure by qualified personnel.

Duration of the study: Anterior nasal swabs will be collected within a three-month period.

Benefits of the study:

MRSA nasal colonization studies will provide a baseline data on MRSA prevalence and antimicrobial resistance among HIV/AIDS patients. This information is necessary for effective management of HIV-infected individuals.

Use of collected material: Bacteria will be isolated from the samples collected from all the study participants. These samples will be destroyed by burning after 2 years. The bacteria may be subjected to genetic studies, and may be preserved in the freezer for future investigations.

Potential hazards of the study:

There may be a slight discomfort when taking the anterior nasal swab specimens from you.

Participant's rights to refuse or withdraw:

If you have read this form, or the content herein has been clearly explained to you, and you have agreed to participate in this study, please note that participation is voluntary and you have the right to withdraw your consent or discontinue participation in the project at any time without penalty. It is also your right to refuse to answer questions you are not comfortable with.

Confidentiality:

All materials collected from you will be coded using numbers and letters. Your privacy will be maintained in all published and written data resulting from the study.

Questions, concerns, or complaints:

If you have any issues relating to this study, please direct them to Prof. Eric Sampane-Donkor (0553527140), Dr. George Antepem Pesewu (0277301300), or Miss Vera Appiah (0503999258), of the Department of Medical Microbiology, SBAHS.

Consent for inclusion: If you agree to be included in this study, please complete the form below;

I .....…… on this day …….. (Day/Month/Year) attest that I understand the explanations given in the consent form and thus give permission to Miss Vera Appiah to include me in the research study

titled "Nasal colonization with methicillin-resistant *Staphylococcus aureus* among sickle cell disease children".

Signature of participant: …….....

Contact address: .....……..

Phone number: …….....

Thumb Print (Where required)