

UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

NASAL COLONIZATION WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS*

AUREUS (MRSA) AMONG HIV-INFECTED CHILDREN

BY

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DECLARATION

I, Fleischer Charles Kotey, declare that the work presented in this thesis is the result of my own research work 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 Sampene-Donkor (SBAHS) and Dr. Nicholas T. K. D. Dayie (SBAHS), and that all references cited in this work have been duly acknowledged.

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DEDICATION

I dedicate this work to my lovely wife, Mrs. Rhoda Kotey, other members of my family, and my friends.

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a public health threat because 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, HIV-infected persons are at a higher risk for colonization with MRSA, and could potentially disseminate the pathogen to other individuals. In Ghana, little is known about MRSA in relation to at-risk populations, such as HIV-infected children.

General aim: The aim of this study was to investigate nasal carriage of *S. aureus* and MRSA among HIV-infected and uninfected children in Accra, including the prevalence, risk factors and antibiogram.

Methodology: This study was cross-sectional, and involved 107 HIV-infected children recruited at the Princess Marie Louis Children's Hospital (PML) in Accra and an equal number of sex- and age group-matched apparently healthy children without HIV infection (control group) recruited from PML and its environs. 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 *mecA* gene. *S. aureus* susceptibility to standard antimicrobial agents was tested by the Kirby Bauer method. Antimicrobials tested included tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), rifampicin (5 µg), cotrimoxazole (1.25 + 23.75 µg), penicillin (10 µg), clindamycin (2 µg), fusidic acid (10 µg), cefoxitin (30 µg), linezolid (10 µg), and vancomycin (30 µg). A pretested structured questionnaire was also used to obtain data on demographic, household, and clinical features of the study participants. Binary logistic regression was performed to identify predisposing factors for *S. aureus* and MRSA carriage among both the HIV-infected and uninfected children.

Results: The carriage prevalence of *S. aureus* and MRSA were 44.9% ($n = 48$) and 5.6% ($n = 6$) respectively among the HIV-infected individuals, and the corresponding values within the control group were 23.4% ($n = 25$) and 0.9% ($n = 1$). HIV infection was significantly associated with *S. aureus* colonization ($p = 0.001$; OR = 2.67), but not MRSA colonization ($p = .055$). The only significant predictor of *S. aureus* colonization in both study groups was absence of colonization with coagulase-negative Staphylococci (CONS): HIV-infected individuals ($p < 0.001$; OR = 0.078); HIV-uninfected individuals ($p < 0.001$; OR = 0.038). Also, the only significant predictor

of MRSA colonization among the HIV-infected participants was regular hand washing with soap ($p = 0.043$; OR = 6.462). None of the variables assessed showed significant associations with MRSA colonization among the HIV-uninfected participants. In both study groups, the *S. aureus* isolates showed no resistance to fusidic acid, linezolid and vancomycin. For the other antimicrobials, the resistance rates recorded in the HIV-infected and uninfected participants respectively were penicillin (98.1% vs. 96%), cotrimoxazole (62.3% vs. 72%), tetracycline (49.1% vs. 40%), rifampicin (30.2% vs. 36%), erythromycin (26.4% vs. 44%), clindamycin (18.9% vs. 52%), gentamicin (15.1 vs. 16%), and cefoxitin (11.3% vs. 4%). The proportion of *S. aureus* isolates that were multidrug resistant (MDR) was 62.3% (33/53) in the HIV-infected group and 80% (20/25) in the control group.

Conclusions: Prevalence of MRSA appears to be generally low among both HIV-infected and HIV-uninfected children in Accra. HIV infection is a risk factor for nasal colonization with *S. aureus* among children in Accra, but may not be for MRSA. Probably due to the low MRSA carriage, determinants of MRSA nasal colonization among the study participants appear to be very limited, and this hardly involves demographic, household, and clinical features. Both HIV-infected and uninfected children are reservoirs of multidrug resistant *S. aureus*, which are entirely susceptible to fusidic acid, linezolid, and vancomycin.

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LIST OF ABBREVIATIONS

AIP.....	Autoinducing peptide
ATCC.....	American Type Culture Collection
BMI.....	Body mass index
CA-MRSA.....	Community-associated MRSA
Cef.....	Cefoxitin
CI.....	Confidence interval
Cln.....	Clindamycin
CONS.....	Coagulase-negative Staphylococci
Cot.....	Cotrimoxazole
CO ₂	Carbon dioxide
DNA.....	Deoxyribonucleic acid
dNTP.....	Deoxyribonucleoside triphosphate
EPRC.....	Ethical and Protocol Review Committee
Ery.....	Erythromycin
FOH.....	Frequency of hospitalization in the past year
Gen.....	Gentamicin
HA-MRSA.....	Healthcare-associated MRSA

HIV.....	Human Immunodeficiency Virus
KBTH.....	Korle Bu Teaching Hospital
LA-MRSA.....	Livestock-associated MRSA
LB.....	Lysogeny broth
MDR.....	Multidrug resistant
MgCl ₂	Magnesium chloride
MSSA.....	Methicillin-susceptible <i>Staphylococcus aureus</i>
MRSA.....	Methicillin-resistant <i>Staphylococcus aureus</i>
N/A.....	Not applicable
OR.....	Odds ratio
PCR.....	Polymerase chain reaction
Pen.....	Penicillin
PML.....	Princess Marie Louis Children's Hospital
PVL.....	Panton-Valentine leucocidin
Res.....	Resistant
Rif.....	Rifampicin
RNase.....	Ribonuclease
Sen.....	Sensitive

SSTI.....Skin and soft tissue infection

TB.....Tuberculosis

Tet.....Tetracycline

TMP-SMZ.....Trimethoprim-sulphamethoxazole

°C.....Degree Celsius

χ^2Chi-square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Staphylococcus aureus causes a broad spectrum of clinically important infections in humans, including meningitis, septicaemia, pneumonia, osteomyelitis and endocarditis (Todar, 2006). Although the pathogen can be carried at several anatomical sites, including the axillae, perineum, vagina, gastrointestinal tract, pharynx, and the skin, its ecological niche is the anterior nares of the nose (Williams, 1963). Among individuals of the general population, some 50% are apparently resistant to *S. aureus* colonization (Hu *et al.*, 1995; Eriksen *et al.*, 1995; Kluytmans *et al.*, 1997; Nouwen *et al.*, 2004). In contrast, 20% of individuals are estimated to be persistent carriers, and the other 30% carry the pathogen intermittently (Kluytmans *et al.*, 1997; Nouwen *et al.*, 2004).

Several factors have been associated with colonization with *S. aureus*, and these include previous intravenous drug use, hospitalization, previous ICU admission, cellulitis at hospital admission, skin ulcers at hospital admission, chronic haemodialysis, non-ambulatory status, diabetes mellitus, neoplasm, presence of a central venous catheter, previous antibacterial therapy, previous colonization or infection with MRSA, and HIV infection (Tacconelli *et al.*, 2004; Cenizal *et al.*, 2008; Kyaw *et al.*, 2012; Farley *et al.*, 2015; Kotpal *et al.*, 2016). Furthermore, *S. aureus* carriers can transfer carrier states to other household members (Peacock *et al.*, 2003; Bogaert *et al.*, 2004).

S. aureus strains that display resistance to methicillin are identified as methicillin-resistant *Staphylococcus aureus* (MRSA); such strains are additionally resistant to all beta-lactam antimicrobials (File Jr. *et al.*, 2012; Ho *et al.*, 2012; Polenakovik & Pleiman, 2013), with reports

on the emergence of resistance to ceftaroline, another beta-lactam antimicrobial, however, occurring quite recently (Mendes *et al.*, 2012; Falagas *et al.*, 2013; Alm *et al.*, 2014; Long *et al.*, 2014; Egyir *et al.*, 2015; Strommenger *et al.*, 2015). The remnants of the *S. aureus* strains are sensitive to methicillin, and are collectively called methicillin-susceptible *Staphylococcus aureus* (MSSA). Because MRSA is primarily a key nosocomial pathogen, it is usually called healthcare-associated MRSA (HA-MRSA) (Moran *et al.*, 2006). Besides HA-MRSA, MRSA strains transmitted in community settings, called community-associated MRSA (CA-MRSA), have been reported (Camargo *et al.*, 2013; Casey *et al.*, 2013; Joore *et al.*, 2013; Loeffler *et al.*, 2013). Strains of the pathogen adapted to livestock, called livestock-associated MRSA (LA-MRSA), also exist (Haenni *et al.*, 2012; Quitoco *et al.*, 2013).

1.2 Problem statement

Back in 1970, MRSA was implicated in just 2% of *S. aureus* infections (O'Toole *et al.*, 1970). By 2006, the pathogen had spread rapidly, and had caused up to 70% of *S. aureus* infections (Hsueh *et al.*, 2002; NNIS, 2004; Tiemersma *et al.*, 2004; Amaral *et al.*, 2005; EARSS, 2006). In Europe, it is implicated in 44% of all healthcare-associated infections (ECDC/EMEA 2009). Worse yet, its infections result in extended hospitalization periods and higher healthcare costs (De Kraker *et al.*, 2011). In the United States for instance, over ninety-four thousand new invasive MRSA infections are estimated to occur annually, resulting in more than eighteen thousand deaths. Hospitalization for infections of MRSA cost \$14,000, as compared to that for all other stays (\$7,600), recording double the duration of hospitalization (Elixhauser & Steiner, 2007; Klevens *et al.*, 2007). Similarly, data from 31 European countries showed over twenty-seven thousand episodes of MRSA blood stream infections which resulted in more than five thousand deaths and more than two hundred and fifty days of hospitalization (De Kraker *et al.*, 2011).

Individuals with HIV infection are at a greater risk for colonization with CA-MRSA (Hidron *et al.*, 2005; Shet *et al.*, 2009; Popovich *et al.*, 2013). Studies across different geographical areas have reported high MRSA carriage prevalence of up to 16% in individuals with HIV infection (Cenizal *et al.*, 2008; Farley *et al.*, 2015; Hassanzadeh *et al.*, 2015; Lemma *et al.*, 2015). This is of major concern, as significant associations exist between MRSA colonization and a higher risk of subsequent infections (Hidron *et al.*, 2005; Wertheim *et al.*, 2005; Popovich *et al.*, 2013; Zervou *et al.*, 2014). Generally, people with HIV infection have an 18-fold increased risk of acquiring CA-MRSA infections (Crum-Cianflone *et al.*, 2007). Furthermore, MRSA-colonized individuals could potentially disseminate the pathogen to other individuals (Imaz *et al.*, 2015), and the occurrence of MRSA in patients is a significant predictor of higher morbidity and mortality (Klein *et al.*, 2007; Burkey *et al.*, 2008; Ganga *et al.*, 2009).

In Ghana, surveillance data have reported MRSA prevalence of 0.3–33% (Donkor & Nartey, 2007; Odonkor *et al.*, 2012; Egyir *et al.*, 2014a; Egyir *et al.*, 2014b; Egyir *et al.*, 2016). Since 2012, there have been several outbreaks of MRSA in Ghana, and the public health threat and substantial untoward economic impact associated with this pathogen places it high on the agenda of public health concerns in the country (Donkor *et al.*, 2018). As part of the overall strategy in addressing the MRSA menace in Ghana, there is a need for surveillance of the pathogen among risk populations, such as individuals with HIV infection.

1.3 Justification of the study

Despite the clinical significance of MRSA nasal carriage in HIV-infected individuals, very few studies have been carried out on the subject in Africa. These few African studies seem to be centred on MRSA prevalence, without identifying the risk factors for carriage (Cotton *et al.*, 2008; Heysell *et al.*, 2011; Olalekan *et al.*, 2012; Egyir *et al.*, 2016). Thus, gaps in knowledge exist concerning

factors that predispose HIV-infected individuals to nasal carriage of MRSA in the sub region. An insight into such risk factors is however essential for tailoring preventive strategies in this risk group (Nguyen *et al.*, 1999). In Ghana, a study evaluating *Streptococcus pneumoniae* carriage in the nasopharynx of HIV-infected children reported a 3.4% MRSA carriage prevalence (Sampane-Donkor *et al.*, 2017). This is noteworthy, as *S. aureus* occurs mainly in the anterior nares, and hence highlights the need for nasal MRSA surveillance among HIV-infected individuals. Such data would enhance the management of *S. aureus* and MRSA infections in HIV-infected children in Ghana.

1.4 General aim

The aim of the study was to investigate *S. aureus* and MRSA colonization among HIV-infected and uninfected children 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 describe the bacterial flora colonizing the anterior nares of HIV-infected and uninfected children visiting the outpatient clinic of Princess Marie Louis Children's Hospital
- To ascertain if HIV infection predisposes to *S. aureus* and MRSA colonization
- To determine the risk factors for colonization with *S. aureus* and MRSA among the HIV-infected and uninfected children
- 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 Characteristics and infections of *Staphylococcus aureus*

S. aureus exist as Gram-positive cocci, mostly in the arrangement of grapelike clusters; they are normally not encapsulated, are non-motile, with spherical cells about 1µm in diameter, and do not form spores (Haghkhah, 2003; Greenwood *et al.*, 2012). Also, *S. aureus* occur as commensals on human skins and animals, and in the respiratory, urogenital, and digestive tracts (Haghkhah, 2003; Acton *et al.*, 2009). The pathogen often spreads from the nose to wounds via the hands, through such events as nose picking (Lowe *et al.*, 1998; Todar, 2006). It produces several pathogenicity factors which act in symphony, causing infections such as meningitis, septicaemia, pneumonia, osteomyelitis, pyomyositis, recurrent furunculosis, impetigo, and botryomycosis (Crossley & Archer, 1997; Lowe *et al.*, 1998). Also, *S. aureus* is mostly implicated as the etiology of infective endocarditis in the developed world (Fowler *et al.*, 2005).

2.2 Virulence factors and pathogenesis of *S. aureus*

Several studies (Lin & Peterson, 2010; Mandal, 2012; Mwambete & Rugemalila, 2015) have revealed that several virulence factors facilitate the adhesion of *S. aureus* to the surface of eukaryotic cells (mucosal epithelium) via a ligand-receptor interaction, promoting the evasion of the immune system, with resultant colonization of the human skin, catalyzing the production of harmful toxic effects to the patient. Mwambete & Rugemalila (2015) posits that basically, two hallmarks of pathogenic bacteria are central to the mechanisms by which they cause diseases, namely invasiveness and toxigenesis. Essentially, microbial infection commences with colonization of host tissues, and after the development of tissue adherence mechanisms and

evasion of host immune system (innate and adaptive immune responses), transits into a diseased state in the host.

Colonization with *S. aureus* could be due to a variety of factors, both of the host and the pathogen (Peacock *et al.*, 2001, 2003). Host proteins, such as fibrinogen, collagen, and keratin are targets for *S. aureus* binding and adherence (Johannessen *et al.*, 2012). As an example, the biocidal agent, triclosan, is used in a variety of products, ranging from toothpaste and soaps, to medical devices, such as sutures and catheters (to prevent microbial infections) (Storch *et al.*, 2004; Halden & Paull, 2005; Jones *et al.*, 2006). However, this biocide has been reported to enhance *S. aureus* binding to those host proteins (fibrinogen, collagen, and keratin), indicating that use of products containing triclosan could promote *S. aureus* colonization (Syed *et al.*, 2014).

Jenkins *et al.* (2015) conducted a study to determine virulence genes contained in two *S. aureus* isolates. The study analyzed 23 putative virulence factors of *S. aureus*, such as secreted toxins, carbohydrate adhesins, plus proteins secreted during the acquisition of metal cations and evasion of the immune system. It was observed that *FhuD*, *sdrC*, *sstD*, *fnbA*, and *hla* consistently increased during the transition of *S. aureus* from colonization to invasion, indicating their vital roles in staphylococcal pathogenesis. The results also highlight the mode with which *S. aureus* transits the expression of virulence genes between commensal and invasive pathogens. The outcome of this study lends credence to findings of previous studies (Powers & Wardenbeurg, 2014; Mwambete & Rugemalila, 2015) regarding the association of genes and proteins of *S. aureus* in several disease states, and provides ground-breaking information on genes that are instrumental to nasal colonization, early bloodstream infection, and thromboembolic lesions.

In their study, Mwambete & Rugemalila (2015) further stated that virulence determinants of *S. aureus* are often transmitted to other organisms of the same species or genera via 1) horizontal

gene transfer; 2) transmission of mobile genetic elements; 3) or other compensatory or adaptive mutations, such as the presence of systems that either activate or repress *CbrA-CbrB* genes (Beceiro *et al.*, 2013; Bernier *et al.*, 2013; Vuotto *et al.*, 2014). These modes form the major genetic mechanism of colonization and spread of virulence and pathogenesis (Mwambete & Rugemalila, 2015).

2.3 *S. aureus* and antimicrobial resistance

In 1941, 90% of staphylococcal infections were susceptible to penicillin; however, within a short while (in 1945), penicillin-resistant *S. aureus* strains emerged (Korn *et al.*, 2001; Shehabel-Din *et al.*, 2003). Penicillin resistance emerged through the production of beta-lactamase by the organisms; the beta lactamase breaks up the beta-lactam ring of the penicillin molecule (Pantosti *et al.*, 2007). Fourteen years later, semi-synthetic penicillins were developed to counteract penicillinase hydrolysis. These semi-synthetic penicillins include methicillin, dicloxacillin, nafcillin and oxacillin. These were introduced (especially methicillin) as therapeutic agents for treating *S. aureus* infections that do not respond to penicillin therapy (Enright *et al.*, 2002; Haghkhah, 2003). Nonetheless, methicillin resistance has also emerged, with organisms displaying this resistance classified as HA-MRSA, CA-MRSA, and LA-MRSA (Camargo *et al.*, 2013; Casey *et al.*, 2013; Joore *et al.*, 2013; Loeffler *et al.*, 2013; Quitoco *et al.*, 2013). It was reported in the UK, that 30-50% of *S. aureus* strains, and over 50% of the coagulase-negative staphylococci were methicillin-resistant. In *S. aureus*, resistance to methicillin occurs when the organism acquires the *mecA* gene, a gene reported to encode *PBP2a* – a novel penicillin-binding protein – which is not bound by methicillin (Crossley & Archer, 1997).

Vancomycin was developed to treat MRSA infections in several countries, such as some Asian countries, Brazil, USA, etc.; however, its therapeutic success was for a short while as resistance to

the drug was reported among MRSA strains (Tiwari *et al.*, 2006; Saha *et al.*, 2008; CDC, 2013; Askari *et al.*, 2013; PAHO/WHO, 2013). Though this phenomenon is rare, and factors contributive to it are difficult to pinpoint, findings from some studies have revealed that resistance to vancomycin is due to the inability of vancomycin to disrupt peptidoglycan fusion (Whitener *et al.*, 2004; Tenover *et al.*, 2004; CDC, 2013; PAHO/WHO, 2013).

CA-MRSA and HA-MRSA are generally characteristically different from each other (Tristan *et al.*, 2007). CA-MRSA frequently causes skin and soft tissue infection (SSTI) and serious infections like osteomyelitis, necrotizing fasciitis and pneumonia, bacteraemia, and endocarditis (Bassetti *et al.*, 2010; Sola *et al.*, 2012). Furthermore, in comparison with HA-MRSA, CA-MRSA characteristically retains susceptibility to majority of the non-beta-lactam antimicrobials, including tetracyclines and cotrimoxazole. In addition, CA-MRSA is often associated with the production of Panton-Valentine leukocidin (PVL), which causes polymorphonuclear leucocyte lysis and tissue necrosis (Tristan *et al.*, 2006; Boyle-Vavra & Daum, 2007).

2.4 Treatment of *S. aureus* infections

Findings of some studies (Markowitz, 1992; Kaka *et al.*, 2006) revealed that linezolid (equivalent to vancomycin), trimethoprim-sulfamethoxazole (TMP-SMZ), doxycycline, clindamycin, and rifampin (combined with other agents) have been used in treating MRSA infections in HIV-infected individuals (Hidron *et al.*, 2010). It must be noted, however, that there are limited cases of randomized controlled trials supporting the efficacy of these drugs (Sakoulas *et al.*, 2003; Forrest & Tamura, 2010; Bartash & Nori, 2017). Linezolid was reported as a very effective aminopenicillin/ β -lactamase inhibitor when treating diabetic foot infections; though there have been cases of resistance to this drug, these have not been clinically significant (Tsiodras *et al.*, 2001; Howe *et al.*, 2002; Lipsky *et al.*, 2004; Morales *et al.*, 2010). However, in a study conducted

by Vinh & Rubinstein (2009), it was discovered that linezolid was associated with myelosuppression, including anaemia and thrombocytopenia (though reversible after discontinuation). Moreover, incidences of neurotoxicity have been reported among patients who had been on linezolid therapy for greater than 28 days (Bressler *et al.*, 2004; Ferry *et al.*, 2005; Vinh & Rubinstein, 2009).

TMP-SMZ, according to the study outcome of Markowitz *et al.* (1992), can inhibit MRSA *in vitro* than most drugs, including linezolid and clindamycin. Similar to vancomycin, it can also be readily used for treating uncomplicated MRSA SSTIs in HIV-infected patients (Hidron *et al.*, 2010). However, there are few reported cases of TMP-SMZ resistance in HIV-infected individuals (Diep *et al.*, 2008).

While some studies have reported that clindamycin is potent for inhibiting PVL and treating MRSA infections in HIV-infected individuals (Daum, 2007; Dumitrescu *et al.*, 2007; Stevens *et al.*, 2007), a study by Shet *et al.* (2009) revealed that almost half the proportion of *S. aureus* isolated from participants in their study (42.9%) were resistant to clindamycin, indicating the need for caution in its use. Rifampin has also been shown to be very potent against MRSA infections in HIV-infected individuals, especially when combined with other agents; however, according to Kaka *et al.* (2006), it could be antagonistic when combined with TMP-SMZ.

2.5 Methicillin-resistant *Staphylococcus aureus* (MRSA)

2.5.1 Epidemiology of MRSA

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).

In Africa, there is limited data on MRSA. One multicentre study which involved five major towns in Africa reported a prevalence of 15% (Breurec *et al.*, 2011). MRSA prevalence of 20% and 45% were reported in Nigeria (Ghebremedhin *et al.*, 2009) and Algeria (Bekkhoucha *et al.*, 2009) respectively. In Ghana, surveillance data have reported MRSA prevalence of 3-33% in the general population (Donkor & Nartey, 2007; Odonkor *et al.*, 2012; Egyir *et al.*, 2014a).

Furthermore, most MRSA strains in Africa share this common trait: they harbour the PVL genes, *lukS/F-pv*, at rates exceeding those of strains from the rest of the world, and this attribute of strains from Africa makes them similar to CA-MRSA strains (0.55% vs. 0.10%) (Breurec *et al.*, 2011; David *et al.*, 2011; Ruimy *et al.*, 2008; Shittu *et al.*, 2011).

2.5.2 Risk factors for *S. aureus* and MRSA colonization

Several risk factors for *S. aureus* and MRSA colonization have been reported, with HIV infection reported as an independent determinant of colonization with both *S. aureus* and MRSA (Miller *et al.*, 2003; Hidron *et al.*, 2005).

Generally, age, gender, close contact among children residing in the same household, homelessness, current antibiotic use, active smoking, and diabetes mellitus have all been reported to predict *S. aureus* nasal carriage (Miller *et al.*, 2003; Bogaert *et al.*, 2004; Nouwen *et al.*, 2004; Miller *et al.*, 2009; Regev-Yochay *et al.*, 2009; Olsen *et al.*, 2012).

Among HIV-infected individuals, factors that reportedly predispose to *S. aureus* colonization include male gender, antimicrobial therapy in hospitalized patients, HIV infection in patients greater than 42 years of age, and prior hospitalization (Seybold *et al.*, 2009; Lemma *et al.*, 2015).

Interestingly, Seybold *et al.* (2009) reported that HIV infection predicts *S. aureus* nasal colonization only in patients above the median age of 42 years, but not in younger patients. They

further demonstrated that history of hospitalization was not a determinant of *S. aureus* colonization in individuals with HIV infection, a report recently corroborated by Lemma *et al.* (2015). Lemma *et al.* (2015) additionally reported that HIV-infected children who had CD4 counts higher than 350 million cells/L were at a higher risk for *S. aureus* carriage.

This seems somewhat surprising, as having lower CD4 counts has been associated with MRSA colonization by several studies (McDonald *et al.*, 2003; Cenizal *et al.*, 2008; Szumowski *et al.*, 2009; Ramsetty *et al.*, 2010; Heysell *et al.*, 2011; Kyaw *et al.*, 2012). Indeed, the relationship between MRSA colonization and having lower CD4 counts is a curious one, as many other studies have failed to show lower CD4 counts as a significant determinant of MRSA colonization (Villacian *et al.*, 2004; Padoveze *et al.*, 2008; Shet *et al.*, 2009).

Additional recognized determinants of MRSA colonization among those with HIV infection include age, lower income, history of pneumonia, lymphoma, prior staphylococcal infection (MSSA or MRSA), skin abscess, not receiving antibiotics, crowding (5 or more members in a household), history of hospitalization of household members for more than twice within twelve months, prior/recent hospitalization, use of street drugs, and incarceration (Cenizal *et al.*, 2008; Kyaw *et al.*, 2012; Zervou *et al.*, 2014; Vieira *et al.*, 2016).

Recent hospitalization, substance abuse, and incarceration are determinants of MRSA colonization among those without HIV infection also, together with such risk factors as area of residence, sexual behaviour, antimicrobial usage within 3 months prior to admission, diagnosis of SSTI at admission, and chronic dialysis (Hidron *et al.*, 2005; Shet *et al.*, 2009; Szumowski *et al.*, 2009; Popovich *et al.*, 2013; Zacharioudakis *et al.*, 2014).

In their studies, Chambers (2001) and Otter & French (2011) reported that patients admitted to rooms that had been inhabited by MRSA-colonized individuals or currently sharing rooms with MRSA-colonized individuals are at significant risk of colonization with MRSA. The results of Chambers' two separate studies involving 1) 8 patients admitted to a room that had been hitherto occupied by MRSA patient, and 2) 472-bed acute-care teaching hospital of which MRSA-positive patients were present (as inpatients), showed that these exposures led to MRSA acquisition. Also, in a study involving 198 patients who were sharing rooms with patients who had unrecognized MRSA colonization over a period of 8 years (1996-2004), Otter & French (2011), reported that 12.6% of the patients acquired MRSA strains that were similar to those of their roommates.

2.5.3 MRSA in Ghana

Studies on MRSA have not been widely conducted in Ghana, leaving a huge gap in knowledge regarding the dynamics of the pathogen in the country. Notably, with the exception of the study that Donkor and Nartey (2007) conducted in Ghanaian children below five years of age, the few MRSA studies conducted in the country have been recent, with the reports spanning from 2012 to date. The study by Donkor and Nartey (2007) itself was not primarily focused on MRSA, but on bacterial organisms in general.

A study by Egyir *et al.* (2016) conducted among HIV-infected individuals reported 8% and 0% nasal carriage prevalence of *S. aureus* and MRSA, with the *S. aureus* isolates belonging to the *spa* types t304, t084, t645, t10828, t311, t774, and t091. Resistance of the *S. aureus* isolates to individual antimicrobials were 100% for penicillin, 40% for tetracycline, and 10% each for rifampicin, fusidic acid, norfloxacin, erythromycin, and sulfamethoxazole trimethoprim, with 30% of the *S. aureus* isolates being multidrug resistant (MDR). The *S. aureus* and MRSA prevalence reported by Egyir *et al.* (2016) contrasts a previous report by the study group (Egyir *et al.*, 2014a)

in the community. That study reported an overall MRSA prevalence of 0.2%, and *S. aureus* nasal carriage prevalence of 21%; the prevalence was significantly higher in the urban study site (28%) than in the rural study site (11%). Apart from the high rates of resistance to penicillin (91%) and tetracycline (25%), the antimicrobial resistance rates recorded for the *S. aureus* isolates sampled from the study participants were generally low, being below 5% against each of fusidic acid, cefoxitin, erythromycin, and norfloxacin. The *S. aureus* isolates belonged to eleven MLST clonal complexes (CC8, CC152, CC45, CC15, CC121, CC97, CC30, CC5, CC508, CC9, and CC707), and were higher in the prevalence of PVL in the urban study site than in the rural study site. An earlier study at the Korle Bu Teaching Hospital (KBTH) reported *S. aureus* carriage prevalence of 13.9% and 23.3% in healthcare workers and inpatients respectively, and MRSA prevalence of 0% and 1.3% respectively in healthcare workers and inpatients (Egyir *et al.*, 2013). The rates of resistance of the *S. aureus* isolates against various antimicrobials were as follows: penicillin (93%), tetracycline (28%) and fusidic acid (12%) cefoxitin (6%), erythromycin (5%), clindamycin (3%), trimethoprim-sulphamethoxazole (3%), norfloxacin (2%), gentamicin (2%), rifampicin (1%) and mupirocin (1%), and linezolid (0%), with 13.3% of the *S. aureus* isolates being MDR. The *S. aureus* isolates belonged to 55 *spa* types, the most common ones being: t084, t355, t127, t002, t537, t008, t304, t630, t861, t1510, t10821, t314, t363, t701, t1476, t2649, t2700, and t10822. Also, healthcare workers ($p = 0.005$) and patients hospitalized for seven days or less ($p = 0.007$) had a higher probability of being *S. aureus* carriers. Another study at KBTH (Sampane-Donkor *et al.*, 2017) investigating of *Streptococcus pneumoniae* carriage in the nasopharynx of HIV-infected children reported *S. aureus* and MRSA carriage prevalence to be 22% and 3.4% respectively. Furthermore, *S. aureus* resistance was highest for penicillin (100%), followed by tetracycline (80.8%), cefuroxime (73.1%), erythromycin (38.5%), ciprofloxacin (19.2%), gentamicin (23.8%)

and cefoxitin (7.7%), with 84.6% of the *S. aureus* isolates being MDR (Sampane-Donkor *et al.*, 2017).

Also in Ghana, Eibach *et al.* (2017) reported that 22.1% of children they sampled in their study were *S. aureus*-colonized, and the carriage prevalence increased to 27.2% in the rainy seasons. Furthermore, 2% of the isolates were demonstrated to be MRSA, 13% displayed resistance to more than two different antimicrobial classes, and 58% carried PVL. The study also revealed that more females (43.7%) were colonized in comparison to fewer males (35.2%). Eibach *et al.*'s (2017) study showed that *S. aureus* prevalence among Ghanaian children is dependent on age, sex and seasonality.

In comparison to the low MRSA prevalence of 0–3.4% referred to earlier, higher MRSA prevalence have been reported by other studies. Donkor & Nartey (2007), referred to earlier, reported a 15% MRSA nasal carriage prevalence among children at KBTH. Likewise, Odonkor *et al.* (2012) and Karikari *et al.* (2017) respectively reported MRSA prevalence of 33.6% in a study involving five hospitals in Accra and 34.8% at the Komfo Anokye Teaching Hospital. The latter two studies however did not focus on colonization, but rather on clinical samples.

The method for screening for MRSA may have accounted for the seemingly higher prevalence reported in the studies of Donkor & Nartey (2007), Odonkor *et al.* (2012) and Karikari *et al.* (2017). These three studies employed phenotypic MRSA screening methods, whereas the others mentioned earlier employed the technique of molecularly detecting the *mecA* gene. Recent reports have however reported the novel *mecC* gene as another molecular mediator of methicillin resistance (Cartwright *et al.*, 2013; Petersen *et al.*, 2013; Becker *et al.*, 2014; García-Garrote *et al.*, 2014; Paterson *et al.*, 2014). Thus studies relying on screening for the *mecA* gene alone as a means

of detecting MRSA, and not in conjunction with phenotypic or *mecC* gene screening could potentially underestimate the MRSA prevalence.

Recently, a study by Saba *et al.* (2017) sought to ascertain the prevalence and antimicrobial susceptibility patterns of *S. aureus* and MRSA within the confines of three hospitals. The study was approached via the collection of 120 swab samples from door handles (78%), stair railings (7%), and other points of contact (8%) at the research sites. In total the prevalence of *S. aureus* was 39%. Of these isolates, 17% were putative MRSA isolates, accounting for an overall prevalence of 0.07%. Among the isolates, one (1) was resistant to oxacillin, cefoxitin, ciprofloxacin, ampicillin, erythromycin, tetracycline, sulfamethoxazole-trimethoprim and streptomycin; five (5) were multi-drug resistant, while the remaining three displayed resistance to two antimicrobials. By implication, Saba *et al.*'s (2017) study underscores the importance of regular and effective cleaning of door handles and other points of contact (such as stair railings, pipe-taps) in hospitals. It also lends credence to the need for further investigation into whether isolates from door handles or stair railings could be an important factor in nosocomial diseases in several hospitals in Ghana.

2.5.4 MRSA nasal colonization and HIV-infected individuals

Most of the studies reporting on MRSA nasal colonization among the HIV-infected are from the developed world (Cenizal *et al.*, 2008; Giuliani *et al.*, 2010; Kyaw *et al.*, 2012; Farley *et al.*, 2015; Hassanzadeh *et al.*, 2015; Imaz *et al.*, 2015; Kotpal *et al.*, 2016). Very few of such studies have been carried out in Africa, providing data on MRSA carriage prevalence and antimicrobial resistance profiles, without reporting on the risk factors for colonization (Amir *et al.*, 1995; Cotton *et al.*, 2008; Heysell *et al.*, 2011; Olalekan *et al.*, 2012; Egyir *et al.*, 2016).

Cenizal *et al.* (2008) assessed MRSA colonization and its risk factors among 146 HIV-infected ambulatory patients attending the HIV clinic of Parkland Hospital in Texas, USA. They reported a nasal carriage prevalence of 10.3% for MRSA, with one participant having axillary colonization also.

Similarly, Hassanzadeh *et al.* (2015) reported a 12.8% MRSA nasal carriage prevalence in a group of HIV-infected individuals in Iran. A parallel report indicated a 9.19% MRSA nasal carriage prevalence among HIV-infected adults of Johns Hopkins University Hospital (Farley *et al.*, 2015).

In stark contrast, Giuliani *et al.* (2010) reported a 0% nasal carriage prevalence of MRSA among HIV-1-infected men who have sex with men in Rome, Italy, even though *S. aureus* nasal carriage prevalence was 23.1%.

Likewise, Imaz *et al.* (2015) reported *S. aureus* carriage prevalence of 32.6% and 2.7% in the anterior nares and pharynx respectively and MRSA carriage prevalence of 1% and 2% at those respective anatomical sites among HIV-infected patients in Barcelona, Spain.

Kyaw *et al.* (2012) reported a 5.1% MRSA nasal carriage prevalence among HIV-infected outpatients in Singapore.

In a study that Kotpal *et al.* (2016) conducted among HIV-infected individuals attending a teaching hospital, they reported 44% and 5.98% respectively for *S. aureus* and MRSA nasal carriage prevalence.

In Ghana, a study by Egyir *et al.* (2016) reported 8% and 0% *S. aureus* and MRSA nasal carriage prevalence, and a 30% nasal carriage prevalence for MDR *S. aureus* isolates. Another study by Sampene-Donkor *et al.* (2017) investigating *Streptococcus pneumoniae* carriage in the nasopharynx of HIV-infected children reported a 3.4% MRSA carriage prevalence.

In neighbouring Nigeria, *S. aureus* and MRSA nasal carriage prevalence of 33% and 16% respectively were reported among the HIV-infected population as opposed to the 21% and 8% in the HIV-uninfected participants. The isolates from the HIV-infected individuals were additionally more resistant to other antimicrobials than were those from those without HIV infection (Olaekan *et al.*, 2012).

2.5.5 Control of MRSA

Generally, measures that are utilized for controlling MRSA in HIV-positive patients include screening for MRSA carriage, improvement of hygiene, isolation of colonized/infected patients, ward closures and, in some situations, decolonization (Muto *et al.*, 2003).

With regard to improvement of hygiene, due to their hands being key vectors for nosocomial transmission of MRSA (Ogston, 1984), healthcare workers (exposed to MRSA) are obliged to maintain a high level of hand hygiene at the points of entry and exit of a MRSA patient's room so as not to transmit it to non-colonized patients. Since the timing of hand hygiene, its efficacy in terms of approaches and compliance by health workers are paramount to achieving any success in this regard, it has been asserted that observance of hand hygiene should be paramount in the following settings: before and after touching a patient or his/her surroundings, before any aseptic procedure, and following exposure to body fluids (Sax *et al.*, 2007).

Generally, key ways through which individuals observe hand hygiene include applying hand sanitizers and/or washing hands with soap (Pittet *et al.*, 1999; Bischoff *et al.*, 2000). However, in light of the reports by Meers (1978), Archibald *et al.* (1997), Klausner *et al.* (1999), and recent reports by Syed *et al.* (2014) and Tetteh-Quarcoo *et al.* (2016), the promotion of hand hygiene as a means of controlling MRSA may require reexamination. According to Meers (1978), frequent hand washing with soap could minimally reduce, or potentially increase, bacterial load of the

hands. Moreover, microbial contamination of soaps could occur (Archibald *et al.*, 1997; Klausner *et al.*, 1999). Also, according to Ojajärvi (1980) and Faoagali *et al.* (1999), at times, hand washing could fail to remove *S. aureus* and MRSA from the hands. The observations made by Meers (1997) and Ojajärvi (1980) seem to be mirrored by the report by Tetteh-Quarcoop *et al.* (2016), who evaluated the microbiological quality of water used for hand washing in preschools; the researchers reported that water from all the schools sampled were heavily contaminated with microbes, mainly, staphylococci. A further twist to the campaign on hand hygiene is the report of Syed (2014), in which triclosan, a component of many soaps and hand sanitizers, was shown to promote *S. aureus* colonization.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study area

This study was conducted at the Princess Marie Louise Children's Hospital (PML) in Accra, the capital city of 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 children's hospital in Ghana. Its HIV clinic, which operates on Tuesdays and Thursdays, attends to about 414 patients – 150 children and 264 adults – per year (personal communication).

3.2 Study design and sampling

The study was cross-sectional, and involved 107 children with HIV infection and an equal number of sex- and age group-matched apparently healthy HIV-uninfected children (control group) recruited between January and July, 2017. The HIV-infected participants were recruited from among HIV-infected children attending the outpatient HIV clinic of the hospital, whilst the control group was recruited from the environs of the hospital. The inclusion criteria for selection of the HIV-infected participants included: being an HIV-infected individual in a steady state, being less than 15 years of age, and being an outpatient.

In the selection of the participants of the control group, the inclusion criteria satisfied were: being HIV-negative, being apparently healthy, and being less than 15 years of age.

In both study groups, the exclusion criteria were: being on antimicrobials (other than cotrimoxazole) two weeks prior to sampling, having known co-morbidities, and inability to determine the HIV status 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 107.

3.3 Specimen and risk factors data collection

Anterior nasal swabs were obtained from the study participants between January and July, 2017. The swab specimens were collected by a trained 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 after 24 hours of incubation at 37°C, inoculated with a sterile loop on blood, chocolate, and MacConkey agars. The blood agar and chocolate agar plates were incubated at 37°C in 5% CO₂, whereas the MacConkey agar plates were incubated aerobically at 37°C. The plates were examined for growth after 18–24 hours.

3.5 Identification of *Staphylococcus aureus*, MRSA, and other bacteria

Following the guidelines by Baron *et al.* (1994), *S. aureus* colonies were presumptively identified from the primary plates based on colonial morphology. These were thereafter touched and streaked onto blood agar plates, and thereafter incubated at 37°C in 5% CO₂ for 18–24 hours. The plates were subsequently examined for growth and identified based on colonial morphology, haemolysis, Gram staining, catalase test and tube coagulase test. *S. aureus* isolates were screened for cefoxitin resistance by the Kirby Bauer method using Mueller-Hinton agar plates. After incubation, isolates that showed zones of inhibition of ≤ 22 mm were interpreted as cefoxitin-resistant, and were identified as MRSA. Cefoxitin-resistant *S. aureus* isolates were screened for carriage of the *mecA* and *nucA* genes.

Other bacterial colonies that grew on the plates were identified by observing their colonial morphologies, reactions to Gram's stain, and their response to standard biochemical tests, including catalase, tube coagulase, oxidase, triple sugar iron, citrate and indole tests.

3.6 Antimicrobial susceptibility testing

Following the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines, susceptibility of *S. aureus* to standard antimicrobials was tested by the Kirby Bauer method, using the following antimicrobials: tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), rifampicin (5 µg), cotrimoxazole (1.25 + 23.75 µg), penicillin (10 µg), clindamycin (2 µg), fusidic acid (10 µg), cefoxitin (30 µg), linezolid (10 µg), and vancomycin (30 µg).

Each test isolate was emulsified in peptone water to create a suspension similar in turbidity to that of 0.5% McFarland standard, with the help of a nephelometer. A sterile cotton swab was dipped into the suspension, and the swab was pressed against the interior walls of the container to drain excess fluid. The resultant swab was thereafter swabbed evenly across the entire surface of a Mueller Hinton agar plate, in three different dimensions, to obtain a semi-confluent growth following incubation. The plates were incubated at 37°C for 18–24 hours, after which the zones of inhibitions around the antimicrobial discs were measured and interpreted according to the breakpoints of CLSI (2017). The control *S. aureus* strain used was ATCC 25923.

3.7 Molecular investigations

Extraction of genomic DNA was done from overnight Lysogeny broth (LB) cultures of the seven cefoxitin-resistant *S. aureus* isolates (for both the HIV-infected and uninfected 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 *mecA* and *nucA* 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 μL , consisting of the genomic DNA (of final concentration 60 $\text{ng}/\mu\text{L}$), PCR water, primers (of final concentration 0.2 μM), Taq polymerase (of final concentration 1.25 $\text{U}/\mu\text{L}$), 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 *mecA* 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 *mecA* PCR, a *nucA* PCR was performed on all the DNA samples, including those of cefoxitin-resistant *S. aureus* descent that showed no bands for the *mecA* 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).

3.8 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, with protocol identification number “**CHS-Et/M.3 – P 4.4/2016-2017**”. 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.

3.9 Data analysis

Data were analyzed with the help of Stata, version 14. Data on the bacterial flora colonizing the study participants, and 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 HIV infection 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

A total of one hundred and seven (107) each of HIV-infected and uninfected children were recruited in this study; their demographic and household features are presented in Table 1. For each group, the males and females comprised 54.2 and 45.8% respectively. The mean BMI recorded for the HIV-infected and HIV-uninfected participants were 16.41 Kg/m² and 17.66 Kg/m² respectively. The mean age of the HIV-infected participants was 6.36 years, and for the HIV-uninfected participants, it was 6.32 years. With the exception of those aged less than 1 year who comprised 1.9% in each study group, the age distribution was similar for the rest of the age groups in both study groups, namely, 1–4years (38.3% in the HIV-infected group and 37.4% in the HIV-uninfected group), 5–9 years (32.7% in the HIV-infected group and 36.4% in the HIV-uninfected group), and > 9 years (27.1% in the HIV-infected group and 24.3% in the HIV-uninfected group). For both groups, a greater proportion of the participants were currently enrolled in school (74.8% for the HIV-infected and 83.2% for the HIV-uninfected group), rarely washed their hands with soap (92.5% in the HIV-infected group and 95.3% in the HIV-uninfected group), and lived in compound houses (86% for the HIV-infected and 84.1% for the HIV-uninfected group). Similarly, the number of individuals per household decreased across < 5 (61.7% for the HIV-infected and 58.9% for the control group), 5–10 persons (35.5% for the HIV-infected and 40.2% for the control group), and 11–20 persons (2.8% for the HIV-infected and 0.9% for the control group), and less than 4% of the participants lived in households which had health worker.

In the HIV-infected group, a greater proportion of the participants were on cotrimoxazole prophylaxis (88.8%) and antiretroviral therapy (89.7%), and indicated that they practice self-medication (58.9%, which is higher than the 41.1% recorded in the HIV-uninfected group). Also, a greater proportion of them had been hospitalized in the past year than those in the HIV-uninfected group (29.9% vs. 11.2%), with 68.9% (as opposed to 100% in the HIV-uninfected group) of that proportion being hospitalized once and 31.1% (as opposed to 0% in the HIV-uninfected group) of them being hospitalized twice. None of the participants in both groups had a chronic skin condition or a history of surgery. However, more participants in the HIV-infected group had a history of pneumonia (4.7% vs. 0.9%) and tuberculosis (6.5% vs. 0%) than the HIV-uninfected group.

The clinical features of the study participants are presented in Table 2 below.

Table 1: Demographic and household characteristics of the study participants

Demographic and household characteristics	HIV-infected		HIV-uninfected	
	Number	%	Number	%
Age				
< 1 year	2	1.9	2	1.9
1–4 years	41	38.3	40	37.4
5–9 years	35	32.7	39	36.4
> 9 years	29	27.1	26	24.3
Gender				
Male	58	54.2	58	54.2
Female	49	45.8	49	45.8
Current school enrolment				
Yes	80	74.8	89	83.2
No	27	25.2	18	16.8
Type of residence				
Self-contained	15	14	17	15.9
Compound	92	86	90	84.1
Number of individuals in household				
< 5	66	61.7	63	58.9
5–10 persons	38	35.5	43	40.2
11–20 persons	3	2.8	1	0.9
Presence of health worker in household				
Yes	4	3.7	3	2.8
No	103	96.3	104	97.2
Hand washing with soap				
Rarely	99	92.5	102	95.3
Often	8	7.5	5	4.7

BMI (HIV-infected) (\bar{X}, SD) = 16.41, 9.06 Kg/m²; BMI (HIV-uninfected) (\bar{X}, SD) = 17.66, 3.87Kg/m²

Table 2: Clinical features of the study participants

Clinical features	HIV-infected		HIV-uninfected	
	Number	%	Number	%
Self-reported self-medication				
Yes	63	58.9	44	41.1
No	44	41.1	63	58.9
Cotrimoxazole prophylaxis				
Yes	95	88.8	0	0
No	12	11.2	107	100
Antiretroviral therapy				
Yes	96	89.7	0	0
No	11	10.3	107	100
History of hospitalization in the past year				
Yes	32	29.9	12	11.2
No	75	70.1	95	88.8
Frequency of hospitalization in the past year				
0	75	70.1	95	88.8
1	22	20.6	12	11.2
2	10	9.3	0	0
Chronic skin condition				
Yes	0	0	0	0
No	107	100	107	100
History of pneumonia				
Yes	5	4.7	1	0.9
No	102	95.3	106	99.1
History of TB				
Yes	7	6.5	0	0
No	100	93.5	107	100
History of surgery				
Yes	0	0	0	0
No	107	100	107	100

Mean CD4 count of HIV-infected participants = 731.20 ± 641.98 cells/mm³

4.2 Bacteria isolated from the HIV-infected and uninfected participants

The bacterial flora present in the anterior nares of the participants of both study groups was diverse, comprising both Gram-positives and Gram-negatives. Two bacterial species alternately dominated in both groups. Among the HIV-infected participants, *S. aureus* was the dominant bacterium, accounting for 44.9%, followed by coagulase-negative Staphylococci (CONS) (43.0%). In the HIV-uninfected group, CONS dominated (55.1%), followed by *S. aureus* (23.4%). Details of bacteria isolated from the study participants are presented in Table 3 below.

Table 3: Bacteria isolated from the study participants

Bacteria isolated	HIV-infected		HIV-uninfected	
	Number	Prevalence (%)	Number	Prevalence (%)
<i>Staphylococcus aureus</i>	48	44.9%	25	23.4%
MRSA	6	5.6%	1	0.9%
CONS	46	43.0%	59	55.1%
<i>Moraxella catarrhalis</i>	4	3.7%	2	1.9%
Diphtheroids	3	2.8%	9	8.4%
<i>Streptococcus viridans</i>	2	1.9%	0	0.0%
<i>Streptococcus pneumoniae</i>	3	2.8%	0	0.0%
<i>Klebsiella</i> spp	3	2.8%	0	0.0%
<i>Klebsiella pneumoniae</i>	1	0.9%	0	0.0%
<i>Pseudomonas aeruginosa</i>	1	0.9%	0	0.0%
<i>Micrococcus</i> spp.	1	0.9%	2	1.9%
<i>Citrobacter koseri</i>	1	0.9%	0	0.0%

It is observed from Table 3 above that the *S. aureus* carriage prevalence was higher among the HIV-infected participants than the HIV-uninfected participants (44.9% vs. 23.4%). This observation was similar for the MRSA carriage prevalence (5.6% vs. 0.9%). The Chi-square test indicated a significant association between HIV infection and *S. aureus* colonization, but not for MRSA colonization, {[*S. aureus* χ^2 (1, $n = 107$) = 10.99, $p = .001$]; [MRSA χ^2 (1, $n = 107$) = 3.69, $p = .055$]}. The odds ratio (OR) for *S. aureus* colonization was found to be 2.67, implying that, the odds of an individual to carry *S. aureus* was about 2.7 times higher if the individual is HIV infected than if not infected.

As observed in Figure 1 below, two of the seven cefoxitin-resistant *S. aureus* isolates screened for carriage of the *mecA* gene did not yield positive results. These isolates, together with the other cefoxitin-resistant *S. aureus* isolates however showed positive results for the presence of the *nucA* gene, confirming them to be *S. aureus* (Brakstad *et al.*, 1992). This is evident in Figure 2 below.

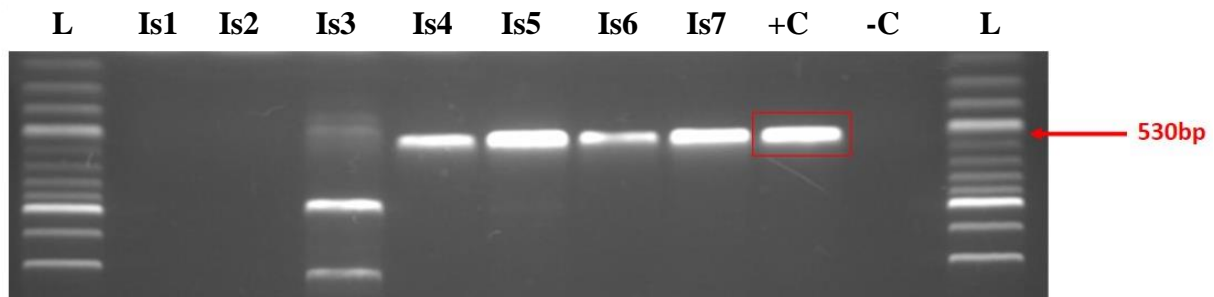


Figure 1: Screening of the cefoxitin-resistant *S. aureus* isolates for *mecA* gene carriage

In the figure, L represents Ladder; Is represents Isolate; +C represents positive control; -C represents negative control

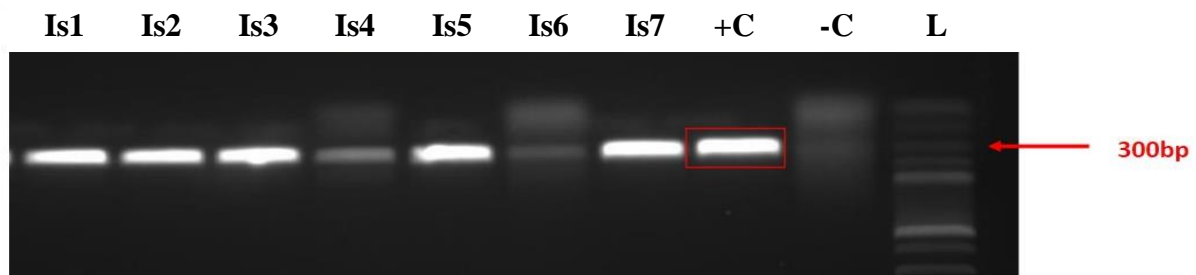


Figure 2: Screening of the cefoxitin-resistant *S. aureus* isolates for *nucA* gene carriage

In the figure, L represents Ladder; Is represents Isolate; +C represents positive control; -C represents negative control

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

In the logistic regression analysis, the main predictor of *S. aureus* colonization was absence of colonization with CONS, in both the HIV-infected and HIV-uninfected participants. This indicates that colonization with CONS is protective of *S. aureus* colonization, regardless of HIV infection status. By inference, absence of colonization with CONS was not a unique risk factor for *S. aureus* colonization among the HIV-infected participants.

Furthermore, the main predictor of MRSA colonization was regular hand washing with soap.

The risk factors for *S. aureus* and MRSA colonization are presented in Table 4 below.

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

Risk factor	HIV-infected		HIV-uninfected	
	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
Colonization with CONS*	0.078 (0.028–0.217)	< 0.001	0.038 (0.008–0.174)	< 0.001
Regular hand washing with soap⁺	6.462 (1.06–39.395)	0.043	N/A	N/A

*associated with *S. aureus* colonization

⁺associated with MRSA colonization

N/A = Not applicable

4.4 Antimicrobial resistance patterns of the *S. aureus* and MRSA isolates of the study participants

The highest proportions of *S. aureus* resistance to the various antimicrobials was recorded for penicillin (98.1% in the HIV-infected and 96% in the control group) and cotrimoxazole (61% in the HIV-infected and 72% in the control group), with no isolate recording resistance against fusidic acid, linezolid, and vancomycin (Figure 5). The proportion of multidrug resistant (MDR) *S. aureus* was 62.3% (33/53) in the HIV-infected group and 80% (20/25) in the control group. In the HIV-infected group, the rates of antimicrobial resistance among the MDR isolates decreased across penicillin (100%, $n = 33$), cotrimoxazole (90.9%, $n = 30$), tetracycline (57.6%, $n = 19$), rifampicin (42.4%, $n = 14$), erythromycin (39.4%, $n = 13$), clindamycin (30.3%, $n = 10$), gentamicin (24.2%, $n = 8$), and ceftiofuran (18.2%, $n = 6$). In the control group, the rates of antimicrobial resistance among the MDR isolates decreased across penicillin (95%, $n = 19$), cotrimoxazole (90%, $n = 18$), clindamycin (55%, $n = 11$), erythromycin (55%, $n = 11$), tetracycline (50%, $n = 10$), rifampicin (45%, $n = 9$), gentamicin (20%, $n = 4$), and ceftiofuran (5%, $n = 1$). The antibiograms of the study isolates for both study groups are presented in Tables 9 and 10.

Table 5: Antibiogram of the *S. aureus* isolates

HIV-infected		HIV-uninfected	
Antibiogram	n	Antibiogram	n
Pen	6	Pen	3
Tet-Pen	7	Pen-Cln	2
Tet-Cot	1	Cot-Pen-Cln	1
Cot-Pen	6	Tet-Cot-Pen	1
Rif-Pen	2	Gen-Cot-Pen	1
Tet-Cot-Pen	9	Ery-Cot-Cln	1
Ery-Cot-Pen	1	Ery-Rif-Pen-Cln	1
Tet-Pen-Cln	1	Ery-Cot-Pen-Cln	1
Rif-Cot-Pen	1	Ery-Gen-Cot-Pen	1
Rif-Pen-Cln	1	Tet-Ery-Cot-Pen	1
Ery-Rif-Cot-Pen	1	Tet-Cot-Pen-Cln	1
Rif-Cot-Pen-Cln	1	Tet-Gen-Rif-Pen	1
Rif-Pen-Cln-Cef	2	Tet-Rif-Cot-Pen	1
Gen-Cot-Pen-Cef	1	Tet-Ery-Cot-Pen	1
Ery-Cot-Pen-Cef	1	Rif-Cot-Pen-Cln	1
Tet-Ery-Cot-Pen	1	Tet-Ery-Cot-Pen	1
Tet-Rif-Cot-Pen-Cln	1	Gen-Rif-Cot-Pen	1
Tet-Gen-Rif-Cot-Pen	1	Ery-Rif-Cot-Pen-Cln	2
Tet-Ery-Gen-Cot-Pen	2	Tet-Cot-Pen-Cln-Cef	1
Tet-Ery-Cot-Pen-Cln	1	Tet-Ery-Rif-Cot-Pen-Cln	2
Tet-Ery-Rif-Cot-Pen	1		
Ery-Gen-Rif-Cot-Pen	1		
Ery-Rif-Pen-Cln-Cef	1		
Ery-Gen-Rif-Cot-Pen-Cln	1		
Tet-Ery-Gen-Rif-Cot-Pen	1		
Tet-Ery-Gen-Rif-Cot-Pen-Cln- Cef	1		

Pen = Penicillin; Tet = Tetracycline; Cot = Cotrimoxazole; Rif = Rifampicin; Ery = Erythromycin; Cln = Clindamycin;

Gen = Gentamicin; Cef = Cefoxitin

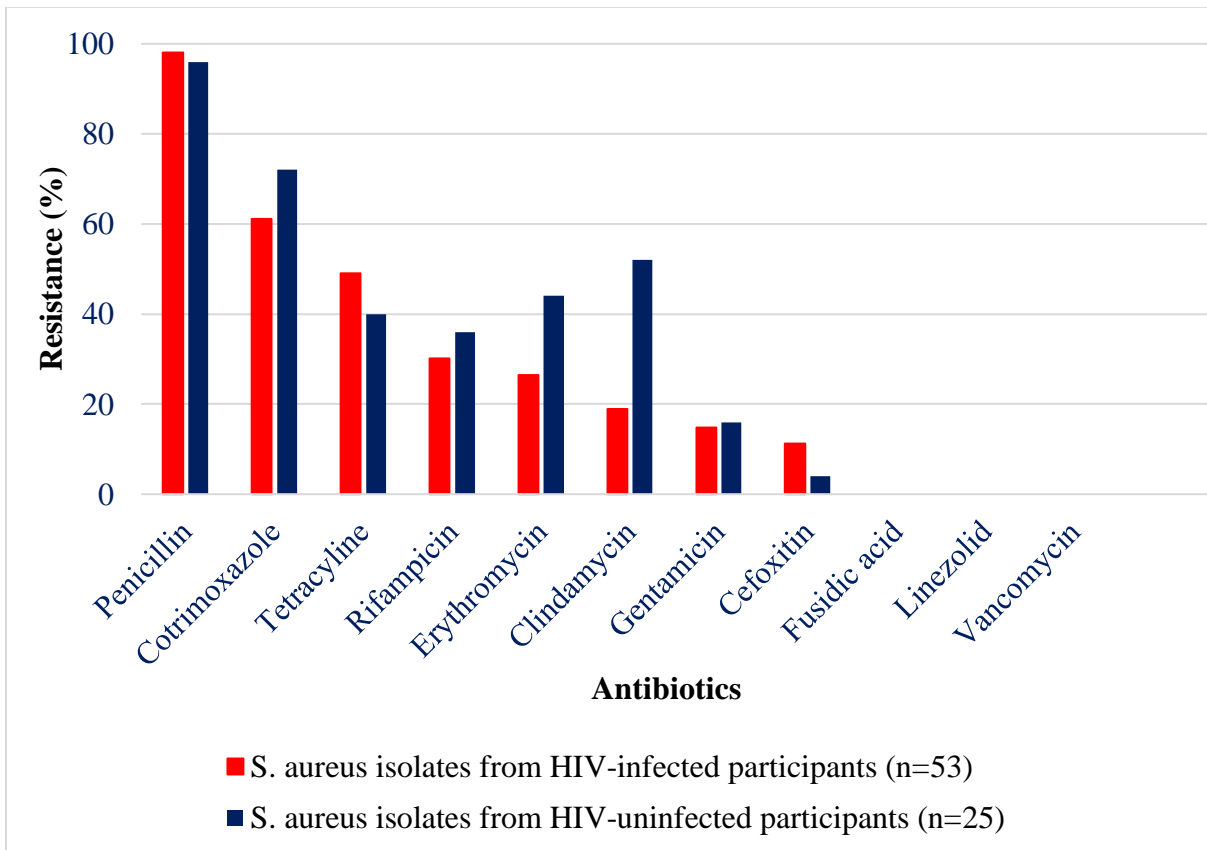


Figure 3: Rates of resistance of *S. aureus* isolates to standard antimicrobials

Table 6: Antimicrobial resistance patterns of the MRSA isolates

Antimicrobials	Is1	Is2	Is3	Is4	Is5	Is6	Is7
Tetracycline	Sen	Sen	Sen	Sen	Res	Sen	Res
Erythromycin	Sen	Res	Sen	Res	Res	Sen	Sen
Gentamicin	Res	Sen	Sen	Sen	Res	Sen	Sen
Rifampicin	Sen	Sen	Res	Res	Res	Res	Sen
Cotrimoxazole	Res	Res	Sen	Sen	Res	Sen	Res
Penicillin	Res	Res	Res	Res	Res	Res	Res
Clindamycin	Sen	Sen	Res	Res	Res	Res	Res
Fusidic acid	Sen	Sen	Sen	Sen	Sen	Sen	Sen
Cefoxitin	Res	Res	Res	Res	Res	Res	Res
Linezolid	Sen	Sen	Sen	Sen	Sen	Sen	Sen
Vancomycin	Sen	Sen	Sen	Sen	Sen	Sen	Sen

Sen = Sensitive; Res = Resistant; Is = Isolate

CHAPTER FIVE

5.0 DISCUSSION

5.1 Bacterial flora colonizing the anterior nares of the study participants

One aspect of this study investigated the bacterial flora colonizing the anterior nares of the HIV-infected and uninfected participants. The bacterial flora present in the anterior nares of the participants in both study groups was diverse, comprising both Gram-positives and Gram-negatives. This diversity is somewhat similar to that reported by Donkor and Nartey (2007) in participants less than five years old, except that in that study, additional organisms (*Streptococcus pyogenes*, *Enterobacter* spp., *Escherichia coli*, *Morganella morgana*, and *Pseudomonas* spp.) were isolated. In the current study, two bacterial species alternately dominated in both groups. Among the HIV-infected participants, *S. aureus* was the dominant bacterium, accounting for 44.9%, followed by coagulase-negative Staphylococci (CONS) (43.0%). In contrast, within the control group, CONS was predominant (55.1%), followed by *S. aureus* (23.4%). This observation in the control group further contrasts the study outcome of Donkor & Nartey (2007) in which the predominant organism was *S. aureus* (49%), followed by CONS (27.4%). These findings additionally contrast earlier findings of Sampane-Donkor *et al.* (2017) who recorded *Moraxella catarrhalis* as the predominant bacterium (39.8%) and CONS as second highest frequently isolated bacterium among HIV-infected children in Ghana. Though the bacterial diversity among the HIV-infected participants in their study was similar to that observed in the present study, *Neisseria meningitidis*, which was isolated in that study was not isolated among the HIV-infected participants in this study. It is noted however, that Sampane-Donkor and his colleagues used swabs obtained from the nasopharynx, which is the ecological niche of the bacterium they primarily

investigated, *Streptococcus pneumoniae*. This difference in the source of specimens from study participants likely accounted for the difference in bacterial dominance and diversity identified in this study compared to that of Sampane-Donkor *et al.*'s (2017).

That *S. aureus* colonization was lower among the HIV-uninfected participants compared to the HIV-infected participants could be as a result of the dominance of CONS. This is probably the case since Paharik *et al.* (2017) have indicated elsewhere that CONS prevent *S. aureus* colonization by inhibiting *agr* quorum sensing in *S. aureus* using its autoinducing peptide (AIP). Moreover, earlier studies by Iwase *et al.* (2010) and Olson *et al.* (2014) demonstrated that *Staphylococcus epidermidis*, one of the organisms categorized as CONS, prevents *S. aureus* colonization.

5.2 The relationship between HIV infection and colonization with *S. aureus* and MRSA

Another objective of this study was to investigate the relationship between HIV infection and *S. aureus* and MRSA colonization. From available literature, this study seems to be the first in Africa to have investigated *S. aureus* and MRSA nasal carriage among children harboring HIV infection in comparison with children without HIV infection. Overall, this study identified HIV infection as a risk factor for *S. aureus* colonization, but not for MRSA colonization.

The results indicate a significantly higher *S. aureus* prevalence among the HIV-infected participants (44.9%), which was significantly higher than the 23.4% prevalence observed in the control arm of the study (44.9% vs. 23.4%; $p = 0.001$). It is also in stark contrast with the 8% prevalence reported in an earlier study involving HIV-infected individuals in Ghana (Egyir *et al.*, 2016). It should be noted however that the participants in Egyir *et al.*'s (2016) study were adults and consisted of both inpatients and outpatients, whereas those in the current study were

ambulatory children. Even though another earlier study among HIV-infected children in Ghana also reported a relatively lower prevalence of 22.0%, it is noted that these researchers used swabs from the nasopharynx (Sampane-Donkor *et al.*, 2017), which is not an ecological niche for *S. aureus*. That the source of swabs used to determine prevalence of *S. aureus* influence the rate of prevalence is attested to by the findings of other researchers in Africa (Lemma *et al.*, 2015). These researchers recorded a higher prevalence for *S. aureus* from the nasal specimens (51.5%) than from the perineal swabs, at rates about half of the values for nasal carriage. Although the *S. aureus* nasal carriage prevalence reported in the current study (44.9%) is lower than what was reported by Lemma *et al.* (2015), it is higher than the 33% and 25% reported by Olalekan *et al.* (2012) and Heysell *et al.* (2013) respectively. Nonetheless, when the *S. aureus* prevalence is considered in totality (44.9% in the HIV-infected and 23.4% in the control group), it does compare well with that reported by Olalekan *et al.* (2012) among adults with HIV infection. In their study, the *S. aureus* nasal colonization prevalence was significantly higher in the HIV-infected group than the control group (33% vs. 21%; $p = 0.0001$). Moreover, it should be noted that the study of Heysell *et al.* (2013) referred to earlier was conducted among TB in-patients with advanced HIV infection. The *S. aureus* prevalence observed among HIV-infected individuals in the current study is consistent with those reported by studies conducted outside Africa too. It is most comparable to the case-control study carried out in India by Kotpal *et al.* (2016) who reported a 44% *S. aureus* nasal carriage prevalence among HIV-infected individuals; this prevalence was significantly higher than that observed in the control group in their study (44% vs. 24%; $p = 0.035$). Results similar to this finding was reported by D'Avila *et al.* (2008) in Brazil. These researchers found that *S. aureus* nasopharyngeal colonization was higher among HIV-1-infected children than those without the infection (45.16% vs. 12.9%, $p = 0.001$). Also, Imaz *et al.* (2015) reported a nasal and

pharyngeal *S. aureus* colonization prevalence of 32.6% and 2.7% respectively among a group of HIV-infected individuals in Spain. Similarly, Datta *et al.* (2008) found the prevalence of *S. aureus* nasal carriage to range between 36.5% and 48% across study sites in Switzerland, further supporting the results of this study.

HIV infection is reported to be an independent determinant of *S. aureus* colonization (Hidron *et al.*, 2010). The situation was no different in this study group, which recorded a higher prevalence of *S. aureus*. That the HIV-infected group recorded a higher prevalence of *S. aureus* than the control group could have been attributed to immunosuppression, except that in this group of children with HIV infection, the mean CD4 count was 731.2 cells/mm³, and that falls within the normal range (500–1500 cells/mm³) (www.hiv.va.gov/patient/diagnosis/labs-CD4-count.asp). Moreover, in this cohort, the level of CD4 counts did not have a significant association with *S. aureus* colonization ($p = 0.678$). However, it has been noted elsewhere that immunosuppression is linked with a higher risk of colonization of PVL-positive *S. aureus* (Schaumburg *et al.*, 2014). Furthermore, *S. aureus* isolates from Africa are known to have a higher prevalence of PVL, a toxin which among other things enhances adherence of *S. aureus* to extracellular matrix, thus facilitating successful colonization.

With regard to nasal carriage of MRSA, the prevalence was also higher in the HIV-infected group than the control group, albeit not statistically significant (5.6% vs. 0.9%; $p = 0.055$). It must be noted, however, that the p value for the observed difference was marginally above the alpha level of 0.05, indicating that an increase in sample size could possibly render the difference observed as significant. Nonetheless, the absence of statistical significance with regard to the difference in MRSA colonization between the HIV-infected and control groups is similar to the observation made in Nigeria by Olalekan *et al.* (2012), whose higher MRSA prevalence among HIV-infected

participants was not significantly different from that recorded within the control group (16% vs. 8%; $p = 0.13$). The observed MRSA nasal carriage prevalence among the HIV-infected is higher than the 0% reported by Egyir *et al.* (2016) among HIV-infected adults, and marginally higher than that reported in the nasopharynx of HIV-infected children (3.4%) by another Ghanaian study (Sampane-Donkor *et al.*, 2017). It is however substantially lower than the 16.8% and 21% reported by Lemma *et al.* (2015) in Ethiopia and Heysell *et al.* (2013) in South Africa respectively. Almost half (42%) of the participants in the study of Lemma *et al.* (2015) were designated to be at WHO stage III and IV HIV infection, an indication of advanced HIV infection. The participants in the study of Heysell *et al.* (2013) were residents of an HIV endemic region, had advanced HIV infection (median CD4+ count = 37 cells/mm³), were co-infected with TB, and were also hospitalized. Notably, hospitalization could promote MRSA dissemination via contaminated surfaces or direct contact with MRSA-colonized patients/health care providers (Vyas *et al.*, 2014). These may account for why the two studies (Lemma *et al.*, 2015; Heysell *et al.* 2013) recorded a markedly higher MRSA prevalence than the current study.

With regard to similar studies conducted outside Africa, the MRSA nasal carriage prevalence observed among the HIV-infected individuals relative to the control group (5.6% vs. 0.9%; $p = 0.055$) is consistent with that reported by Kotpal *et al.* (2016) in India (6% vs. 2%). Unfortunately, the researchers failed to report whether or not this observed difference in prevalence was statistically significant. The MRSA prevalence observed in the current study group is however higher than the 0–1% reported by Giuliani *et al.* (2010) and Imaz *et al.* (2015) among HIV-infected individuals in Italy and Spain respectively. Yet, it is lower than the 9.19–10.3% reported by Farley *et al.* (2015) and Cenizal *et al.* (2008) among individuals with HIV infection in USA.

Although it has been reported in earlier studies that persons harboring HIV infection are predisposed to MRSA colonization (Hidron *et al.*, 2005; Shet *et al.*, 2009; Popovich *et al.*, 2013; Zervou *et al.*, 2014), this study found no such association. The reason for this observation is unclear, but could be multifactorial: The study participants were in steady state, and their health had not deteriorated to an extent that would require hospitalization; in fact, their mean CD4+ count was 731.2 cells/mm³, 89.7% of the participants were on antiretroviral therapy, and only 29.9% of them had been hospitalized in the year prior to the commencement of the study. Antiretroviral therapy has been demonstrated to counter some of the immunologic abnormalities that predispose HIV-infected persons to colonization with MRSA (Imaz *et al.*, 2010). Moreover, lower CD4 counts, prior hospitalization and hospitalization have all been associated with a higher rate of MRSA colonization (Vyas *et al.*, 2014; Zervou *et al.*, 2014).

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

This study additionally aimed to identify specific risk factors that could improve insight on the dynamics of *S. aureus* and MRSA nasal colonization among HIV-infected children in Ghana. Among the independent variables evaluated as risk factors for *S. aureus* and MRSA colonization, absence of colonization with CONS was identified as a determinant of *S. aureus* carriage, regardless of HIV infection status. Colonization with CONS appears to be protective of *S. aureus* colonization. This finding is supported by the reports of Iwase *et al.* (2010), Olson *et al.* (2014), and Paharik *et al.* (2017) referred to earlier.

The other independent variables evaluated were demonstrated not to be predictors of *S. aureus* colonization. Elsewhere, some of these variables, such as age, gender, and history of hospitalization, have been identified as predictors of *S. aureus* colonization (Miller *et al.*, 2003; Bogaert *et al.*, 2004; Nouwen *et al.*, 2004; Miller *et al.*, 2009; Regev-Yochay *et al.*, 2009; Seybold

et al., 2009; Lemma *et al.*, 2015). The inability of these variables to predict colonization in this study could possibly be as a result of variations of the current population from that of the populations in other studies.

With regard to MRSA colonization, none of the variables were demonstrated to be predictive of colonization among the HIV-uninfected individuals. This was expected, given that only one HIV-uninfected participant was MRSA-colonized. Regular hand washing was shown to be a significant predictor of MRSA colonization among the HIV-infected participants. HIV-infected participants who regularly washed their hands with soap were 6.462 times more likely to be MRSA-colonized. This finding is interesting, as good hygiene practices have been promoted as a means of controlling the spread of infections of several pathogens for many years (Khan, 1982; Garner & Favero, 1986; Stanton & Clemens, 1987; Wilson *et al.*, 1991; Pinfold & Horan, 1996; Widmer, 2000; Freeman *et al.*, 2014).

Nonetheless, the finding that regularly washing the hands with soap is a determinant of MRSA colonization is supported by a recent study by Syed *et al.* (2014). That study demonstrated that triclosan, a biocidal component of soaps and many skin care products, was commonly present in nasal secretions, and facilitated *S. aureus* binding with nasal proteins, such as keratin, fibronectin, and collagen. Consequently, hand washing with soaps that contain triclosan could potentially predispose individuals to *S. aureus* and MRSA carriage. Moreover, the study by Tetteh-Quarcoo *et al.* (2016) highlights the potential for dissemination of the pathogen through water of poor microbiological quality, and this may have contributed to the finding that regular hand washing with soap promotes *S. aureus* and MRSA carriage.

5.4 Antimicrobial resistance patterns of the *S. aureus* and MRSA isolates

Finally, this study investigated the antimicrobial resistance patterns of *S. aureus* and MRSA isolated from the HIV-infected and uninfected participants. No resistance was recorded for fusidic acid, linezolid, and vancomycin among the *S. aureus* isolates in both the HIV-infected and the control groups. This rate of fusidic acid resistance contrasts the higher rate reported in another cohort of HIV-infected individuals (10%) (Egyir *et al.*, 2016) and another study in the general population (12%) (Egyir *et al.*, 2013), but consistent with the low rates of 0–2.4% reported by Egyir *et al.* (2014a), Egyir *et al.* (2014b), and Dekker *et al.* (2016). Moreover, the rate of linezolid and vancomycin resistance recorded for *S. aureus* in this study are consistent with those reported in earlier studies (Egyir *et al.*, 2013; Dekker *et al.*, 2016). These three antimicrobials are not widely available in the country, are quite expensive, and are rarely used or abused. Hence the low rates of resistance recorded against them in this study was expected.

The HIV-infected group recorded higher resistance rates for penicillin, tetracycline, and cefoxitin than did the control group. This finding corroborates that of the study of (Olalekan *et al.*, 2016) in which the *S. aureus* isolates carried by the HIV-infected participants recorded higher rates of antimicrobial resistance than did the control. However, the control group carried *S. aureus* isolates with higher antimicrobial resistance rates for five antimicrobials (erythromycin, cotrimoxazole, rifampicin, clindamycin, and gentamicin). To put the higher resistance rates recorded against five antibiotics within the control group in perspective, it must be noted that the percentage resistance recorded within the control group was based on a total number of twenty-five (25) *S. aureus* isolates, whereas that in the HIV-infected group was based on a total of fifty-two (52) *S. aureus* isolates. This may be the reason behind the seemingly higher resistance rates to erythromycin,

cotrimoxazole, rifampicin, clindamycin, and gentamicin within the control group as opposed to the HIV-infected group.

In both study groups, the *S. aureus* isolates were > 95% penicillin resistant, > 60% cotrimoxazole resistant, \geq 40% tetracycline resistant, > 25% erythromycin and rifampicin resistant, > 20% clindamycin resistant, > 15% gentamicin, and \geq 4% cefoxitin resistant. These rates of antimicrobial resistance are comparable to what have been observed by other researchers (Olalekan *et al.*, 2012; Kotpal *et al.*, 2015; Dekker *et al.*, 2016; Egyir *et al.*, 2016; Sampane-Donkor *et al.*, 2017).

The high rates of resistance to tetracycline, cotrimoxazole, and penicillin in both groups is not surprising, as for several years, high rates of resistance have been displayed against these drugs by several microorganisms – clinical isolates and normal intestinal flora from healthy individuals (Donkor & Nartey, 2007). In fact, cotrimoxazole has been used as prophylaxis among HIV-infected individuals for years.

Rifampicin resistance was higher in *S. aureus* isolated from the HIV-infected individuals in this study than those reported among another HIV-infected cohort (10%) (Egyir *et al.*, 2016), and in the general population (0–1%) (Egyir *et al.*, 2013; Egyir *et al.*, 2014b). The occurrence of rifampicin resistant *S. aureus* isolates in this cohort at such a high rate (42%) has serious clinical implications as rifampicin is part of the therapeutic agents used in treating MRSA infections and infections of *Streptococcus pneumoniae*, a pathogen which has been associated with immunosuppression (Forrest & Tamura, 2010). Furthermore, if these circulating resistant strains were to transfer their rifampicin resistance attribute to any of the organisms designated as part of the *Mycobacterium tuberculosis* complex, it could potentially negatively impact TB chemotherapy, for two main reasons. First, rifampicin is a major frontline drug used in the

management of TB infections (Mitchison, 2000). Second, HIV-infected individuals are prone to TB infections (Egyir *et al.*, 2016).

Resistance to ceftazidime is a marker for resistance to methicillin, also a predictor of multidrug resistance, including resistance to beta-lactam antimicrobials. The rate of resistance demonstrated against ceftazidime by the *S. aureus* isolates among the HIV-infected in this study is comparatively higher than the 0–7.7% reported in other cohorts of HIV-infected individuals (Egyir *et al.*, 2016; Sampane-Donkor *et al.*, 2017) and other studies in the general population (1.6–6%) (Egyir *et al.*, 2014a; Egyir *et al.*, 2014b). Of the seven ceftazidime-resistant *S. aureus* isolates, five (four in the HIV-infected and one in the control group) demonstrated positive results for carriage of the *mecA* gene, which in *S. aureus*, predominantly confers resistance to methicillin (Crossley & Archer, 1997). The other two ceftazidime-resistant isolates (both in the HIV group) did not show positive results for *mecA* gene carriage. Hence, methicillin resistance in these isolates may be mediated by the novel methicillin resistance gene, the *mecC* gene, rather than the initially reported *mecA* gene. In fact, *mecC*-mediated methicillin resistance is emerging, and has been widely reported (Cartwright *et al.*, 2013; Petersen *et al.*, 2013; Becker *et al.*, 2014; García-Garrote *et al.*, 2014; Paterson *et al.*, 2014).

Multidrug resistant *S. aureus* isolates were higher in number in the HIV-infected group ($n = 33$) than the control group ($n = 20$), but when considered proportion-wise, they were higher in the control group than in the HIV-infected individuals (80% vs. 62.3%). The MDR isolates were predominantly resistant to penicillin (100% in the HIV-infected group and 95% in the control group) and cotrimoxazole (90.9% in the HIV-infected group and 90% in the control group). The rate of MDR *S. aureus* isolates recorded in this study is within the range of 30–84.6% reported in other HIV-infected cohorts (Egyir *et al.*, 2016), but higher than what have been recorded in studies

involving the general population (6–35.7%) (Egyir *et al.*, 2014a; Egyir *et al.*, 2014b; Dekker *et al.*, 2016).

These rates of multidrug resistance are alarming, though not surprising. In Ghana and many other developing countries, antimicrobials could be obtained without any prescriptions (Lee *et al.*, 1991; Adu-Sarkodie, 1997; Reeves, 2007). Also, self-medication with antimicrobials is common practice, with the prevalence reportedly being 70–75% (Adu-Sarkodie, 1997; Donkor *et al.*, 2012a). In the study by Donkor *et al.* (2012a), they reported that about half of the study respondents did not complete an antimicrobial course. Furthermore, antimicrobial misuse is high in animal husbandry in Ghana (Donkor *et al.*, 2012b), and occurs at the hospital level too (Bosu & Ofori-Adjei, 2000).

Since this study was carried out in a hospital setting, these highly resistant isolates could potentially be culprits in nosocomial infections, and the resistant states could be transferred to other organisms. They could also be transmitted to non-carriers, who could subsequently further disseminate them. This is because a greater proportion of the study participants were currently enrolled in school (74.8% for the HIV-infected and 83.2% for the comparison group) and lived in compound houses (86% for the HIV-infected and 84.1% for the comparison group). These high rates of resistance are therefore of public health concern, and need to be addressed, as community outbreaks with such highly resistant organisms could be imminent.

Nonetheless, the high rates of penicillin and tetracycline resistance do not have much clinical relevance, since these two antimicrobials are not routinely used in treating *S. aureus* infections. However, the high rate of cotrimoxazole resistance is of high clinical significance, as the antimicrobial is routinely administered to HIV-infected individuals as prophylaxis. The absence of resistance to fusidic acid, linezolid, and vancomycin, even in MRSA isolates, suggests that these

antimicrobials remain effective therapeutic options for managing any MRSA infections that may develop in this cohort of HIV-infected children.

CHAPTER SIX

6.0 CONCLUSIONS, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusions

The main conclusions of the study are as follows:

Among the HIV-infected children, the bacteria that predominantly colonized the anterior nares were *S. aureus* (44.9%) and coagulase negative staphylococci (43%); in the control group, the predominant bacteria were coagulase negative staphylococci (55.1%) and *S. aureus* (23.4%).

Furthermore, HIV infection was a significant risk factor for *S. aureus* colonization among children in Accra, but may not be for MRSA colonization, with the odds of individuals to be colonised with *S. aureus* being about 2.7 times higher if the individual was HIV-infected (OR = 2.67).

Also, absence of colonization with CONS was a risk factor for *S. aureus* colonization, regardless of HIV infection status.

Moreover, regular hand washing with soap was significantly associated with MRSA colonization among the HIV-infected participants, which could possibly be due to triclosan in soaps/detergents used for hand washing.

Finally, both HIV-infected and uninfected children are reservoirs of multidrug resistant *S. aureus*, which are entirely susceptible to fusidic acid, linezolid, and vancomycin.

6.2 Recommendations

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

First, since this cohort of HIV-infected children had a high prevalence of *S. aureus* colonization, monthly nasal mupirocin application (Gordon *et al.*, 2010) could be included in the management

of this cohort of patients to reduce their rates of colonization and hence their risk of acquiring *S. aureus* infections.

Furthermore, additional studies need to be carried out in the study area, with a specific focus on molecular characterization of *S. aureus* clones carried by HIV-infected and uninfected individuals. Other studies could also be conducted to further investigate the role hand washing with soap plays in MRSA colonization.

Finally, the high rates of *S. aureus* resistance to cotrimoxazole indicates that the use of this antimicrobial as prophylaxis in HIV-infected participants needs reevaluation.

6.3 Limitations

The limitations of the study include the following:

This study was cross-sectional in nature, so does not distinguish between persistent and intermittent carriers. Furthermore, some of the intermittent carriers may have been missed, given that intermittent carriers were probably not colonized at the time of sampling.

To add to, the *S. aureus* and MRSA colonization prevalence reported may have been underestimated, since only one anatomical site, the anterior nares, was sampled. Some studies have reported that nasal screening alone could underestimate carriage by at least one-third (Zervou *et al.*, 2014).

Moreover, although children with known co-morbidities were excluded from the study, there may have been some study participants with unknown co-morbidities.

Also, even though the *S. aureus* isolates were all sensitive to vancomycin, this was not confirmed by determination of the minimum inhibitory concentration of vancomycin.

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APPENDIX I

RESEARCH CONSENT FORM

Investigator: Mr. Fleischer Charles Kotey

Institution: Department of Medical Microbiology, SBAHS.

Research title: Nasal colonization with methicillin-resistant *Staphylococcus aureus* among HIV-infected 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, particularly, the HIV-infected, because their immune system is less able to fight diseases. Also, infections caused by MRSA are difficult to treat. It is therefore important to investigate nasal colonization of MRSA in HIV-infected 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 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 (0547844664), Dr. Nicholas T. K. D. Dayie (0208449415), or Mr. Fleischer Charles Kotey (0265662494), 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 Mr. Fleischer Charles Kotey to include my ward in the

research study titled “Nasal colonization with methicillin-resistant *Staphylococcus aureus* among HIV-infected children”.

Signature of participant’s guardian:

Contact address:

Phone number:



Thumb print (where required)

APPENDIX II

RESEARCH ASSENT FORM

Investigator: Mr. Fleischer Charles Kotey

Institution: Department of Medical Microbiology, SBAHS.

Research title: Nasal colonization with methicillin-resistant *Staphylococcus aureus* among HIV-infected 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, particularly, the HIV-infected, because their immune system is less able to fight diseases. Also, infections caused by MRSA are difficult to treat. It is therefore important to investigate nasal colonization of MRSA in HIV-infected 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 (0547844664), Dr. Nicholas T. K. D. Dayie (0208449415), or Mr. Fleischer Charles Kotey (0265662494), 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 Mr. Fleischer Charles Kotey to include me in the research study titled "Nasal colonization with methicillin-resistant *Staphylococcus aureus* among HIV-infected children".

Signature of participant:

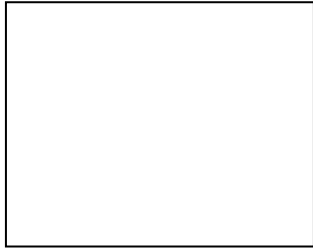
.....

Contact address:

.....

Phone number:

.....



Thumb Print (Where required)

APPENDIX III

MRSA PROJECT: QUESTIONNAIRE FOR ANALYSIS OF RISK FACTORS

SECTION A: BIODATA/DEMOGRAPHY

- | | |
|--------------------------------|-------------|
| 1. Patient ID | |
| 2. Age | |
| 3. Sex | Male/Female |
| 4. Height | |
| 5. Weight | |
| 6. CD4 count | |
| 7. Are you enrolled in school? | Yes/No |

SECTION B: HOUSEHOLD CHARACTERISTICS

- | | |
|--|----------------------|
| 8. Type of Residence: | Residential/Compound |
| 9. How many people live in your house? | |
| 10. How frequently do you wash your hands with soap? | Rarely/Often |

SECTION C: MEDICAL HISTORY

- | | |
|--|--------|
| 11. Do you self-medicate? | Yes/No |
| 12. Are you on cotrimoxazole prophylaxis? | Yes/No |
| 13. Are you on antiretroviral drugs? | Yes/No |
| 14. Have you been hospitalized within the past year? | Yes/No |
| 15. If your answer to (14) is yes, how many times? | |
| 16. Do you have any underlying disease condition? | Yes/No |

17. If your answer to (16) is yes, what condition is it?
18. Do you have a chronic skin condition Yes/No
19. Have you had a history of pneumonia? Yes/No
20. Do you have a history of tuberculosis? Yes/No
21. Do you have a history of surgical procedures? Yes/No
22. Is any member of your household a health worker? Yes/No