KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

Epidemiology of Nasal Carriage of *Staphylococcus Aureus* among hospitalised children at Agogo Presbyterian Hospital, Ghana

By

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OCTOBER, 2016

EPIDEMIOLOGY OF NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*

AMONG HOSPITALISED CHILDREN AT AGOGO PRESBYTERIAN

HOSPITAL, GHANA

A thesis submitted to the Department of Clinical Microbiology in partial fulfillment of the requirements for the award of master of philosophy (mphil.) degree in Clinical Microbiology

in the department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, KNUST, Ghana

By

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OCTOBER, 2016

DECLARATION

I declare that this thesis is my own work towards the award of an MPhil in Clinical Microbiology. This work has not been submitted for the award of any other degree in any university, except where due acknowledgement has been made in the text.

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ACKNOWLEDGEMENT

I am grateful to the almighty God for the favours throughout this research work. My profound gratitude goes to my supervisors and mentors: Dr. Alex Owusu-Ofori, of the Department of Clinical Microbiology, KNUST, Prof. Ellis Owusu-Dabo of Kumasi Centre for Collaborative Research (KCCR), Dr. Daniel Eibach of Bernhard Nocht Institute, Germany and Dr. Michael Nagel of KCCR, Ghana for their supervision, intellectual guidance and above all their magnanimous words of inspiration since the conception of this project, during my laboratory work and the presentation of this thesis. Thank you for your mentorship. An appreciation also goes to my family for the support and prayers throughout my studies. I am also grateful to Dr. Benedictk Hogan, Mr. Kennedy Gyau Boahen, Mrs. Charity Wiafe Akenten, the entire industrious team of AG May and the staff of the Agogo Presbyterian Hospital and KCCR for their technical support. The mentorship and sponsorship of Prof. Juergen May and the German Center for Infection Diseases Research (DZIF) towards this research and my studies are hereby acknowledged.

I gratefully acknowledge Prof. Gabriele Bierbaum of Universitätsklinikum Bonn Institut für Medizinische Mikrobiologie und Immunologie, Germany for her gracious collaboration which allowed me to carry out the molecular aspect of this research in her laboratory. I deeply appreciate the support of the Institute and the laboratory staff especially Mike Gajdiss for the training.

DEDICATION

This work is dedicated to the Almighty God who favoured me greatly.

ABSTRACT

Staphylococcus aureus is an important human pathogen. Nasal carriage of *S. aureus* is a major risk factor for subsequent invasive *S. aureus* infections and transmission in the environment. However, little is known about the prevalence, virulence and diversity of *S. aureus* among children admitted to hospitals in Ghana. This study aimed to describe the epidemiology of nasal carriage of *S. aureus* among hospitalised Children at the Agogo Presbyterian hospital. Nasal swabs were collected from hospitalised children. *S. aureus* and MRSA were identified and characterized by phenotypic and genotypic methods. *S. aureus* was confirmed by *nuc*A PCR and MRSA was by *mec*A and *mec*C PCR. Antimicrobial susceptibility test was performed by the Kirby-Bauer disk diffusion method. PCR was used to amplify genes encodng for Panton-Valentine Leukocidin (PVL) toxin. Typing of the isolates was done by the staphylococcal protein A (spa*)* typing. Out of the 545 nasal swabs, 22.0% (n=120) *S. aureus* were isolated. High level of resistance against penicillin (96.7%) and tetracycline (52.5%) was observed. Gentamycin, trimethoprim/sulfamethoxazole, clindamycin and erythromycin recorded 99.2%, 97.5%, 94.2% and 86.7% sensitivity respectively. All the isolates were sensitive to linezolid and teicoplanin. Of all the *S. aureus* isolates, 1.7% (n=2) were MRSA. The MRSA strains were resistant to penicillins, cefoxitin and tetracycline. High prevalence of PVL (57.5%) was noted among the *S. aureus*. Thirty-five *spa* types were identified; predominant *spa* types were t355 (20%), t084 (14.9%) and t939 (10.7%). The *spa* types for the MRSA were t1096 and t4454. Two novel *spa* types (t15727 and t15728) were identified and submitted to the *spa* database. This study has shown that a substantial number of the hospitalized children were nasal carriers of *S. aureus* with low prevalence of MRSA and high prevalence of PVL. The high prevalence of *S. aureus* and high rate of resistance to penicillin and tetracycline coupled with high prevalence of PVL is a major threat to public health in Ghana. This study has therefore provided data on the nature of nasal carriage of *S. aureus* during hospital admission and could guide policy on infectious diseases prevention and the monitoring of the evolution of *S. aureus* strains in Ghana and globally over time.

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CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND

Staphylococcus aureus (*S. aureus*) is a Gram-positive, catalase and coagulase-positive cocci in clusters bacterium. It is often found on the skin, respiratory tract and in the nose usually unnoticed (Chambers and Deleo, 2009). According to Nagel *et al*., (2013), *S. aureus* is a well-known pathogen that has the potential of causing serious infections in humans and animal. The infections caused by this pathogen range from relatively simple skin and soft tissue infections (SSTI) which include folliculitis, pimples, furunculosis to invasive diseases such as osteomyelitis, bactereamia, pneumonia, toxic shock syndrome, deep abscesses, food poisoning and endocarditis (David and Daum, 2010).

Pathogenic *S. aureus* strains are able to cause infections mostly as a result of the expression of a wide range of virulence factors such as adhesion structures, potent protein toxins and enzymes. These virulence factors facilitate the bacteria in evading the innate immune defense and antibiotics, which mediates the survival of the bacteria and tissue invasion at the site of infection (Gordon and Lowy, 2008).

Staphylococcus aureus colonizes about one third of the human population usually on the skin and in the nose. The nasal cavity is demonstrated to be the most predominant site of colonisation (Chambers and Deleo, 2009). According to Butterly *et al*., (2010), nasal carriage of *S. aureus* is usually asymptomatic. However, this carriage is identified as a major risk factor for subsequently acquirimg *S. aureus* infection. Colonized individuals are more proned to be infected with their own colonizing strains (Butterly *et al*., 2010). In fact, *S. aureus* colonization is usually not a health concern in a healthy individual, but infection may occur in immune compromised individuals or if there is a breach of the

protective barrier of the nose and the skin which may lead to invasion into other sterile sites from the site of carriage (Boucher *et al*., 2010). Historically, *S. aureus* infections were treatable with common antibiotics including penicillin until the emergence of penicillin resistant strains two years after its introduction. Currently, 80%–95% of *S. aureus* strains are resistant to penicillin and this resistance is mediated by the enzyme penicillinase (or β-lactamase) (Sakoulas and Moellering, 2008). Emergence of methicillin resistant *S. aureus* (MRSA) is a major health problem worldwide because of the increased morbidity and mortality rate associated with it due to the limited therapeutic options. Resistance to methicillin is usually mediated by the *mec*A gene which is found on a staphylococcal chromosome *mec* (*SCCmec*) that codes for an altered penicillin binding protein (PBP2a). This PBP2a has a low affinity for beta-lactam antibiotics (Chambers and Deleo, 2009). In attempt to control MRSA, glycopeptide antibiotics; vancomycin and teicoplanin and other new classes of antibiotics are deployed against invasive MRSA. However, frequent and inappropriate use of these antibiotics have also led to incidence of Vancomycin intermediate *S. aureus* (VISA) and Vancomycin resistance *S. aureus* (VRSA) (Sakoulas and Moellering, 2008). Recently, an altered form of *mec*A gene has also been described to be situated on a SCCmec XI element. It is named *mec*C (Monecke *et al*., 2013).

The development of resistance to antibiotics limits treatment options and increases treatment failure. It has been shown that the genetic determinants for resistance to antibiotics are located on plasmids, chromosomal DNA or on transposable elements. The loss or acquisition of these genetic determinants contributes to changes in the resistance patterns in a particular strain (Lindsay, 2010).

Combined data from in-vitro susceptibility tests are used to monitor resistance levels to antibiotics, which in turn influences health care prescribing policies and the development

of new antimicrobial agents in the future. Susceptibility profile of *S. aureus* also aids in the correct implementation of antibiotic regimens for *S. aureus* infections (Jorgensen and Ferraro, 2009). MRSA strains were noted to be associated with health care settings and were known as hospital-acquired/associated MRSA (HAMRSA). Chambers and Deleo, (2009) indicated that, there are established risk factors for acquiring HA-MRSA. These predisposing factors include surgery, indwelling percutaneous medical devices and catheters, long-term residence in a healthcare facility, dialysis and recent hospitalization. These HA-MRSAs are usually multidrug resistant and belong to staphylococcal chromosome *mec* (*SCCmec*) types I, II or III. However, MRSA infections have been found among healthy individuals in the community who are not exposed to these known risk factors for acquiring MRSA infections. These infections are often referred to as community-acquired or associated MRSA (CA-MRSA) infections. These CA-MRSAs contain SCC*mec* types IV and V and they are susceptible to most non β-lactam antibiotics. They often carry a toxin called Panton-Valentine leukocidin (PVL), which is associated with skin and soft tissue infection (SSTI) and necrotising pneumonia (David and Daum, 2010). Since its first description in 1961, different MRSA strains are spreading worldwide although, their prevalence varies from different countries (Jevons, 1961; Breurec *et al*., 2011). Studies have identified *S. aureus* Strains t084 and t355 to be widely distributed in Ghana and most African countries (Egyir *et al*., 2014a; Breurec *et al*., 2011).

The prevalence of *S. aureus* varies with geographical location, the studied population and the type of hospital. High prevalence has been recorded in hospitals in US,

Southern European countries, South America and Asia (Wertheim *et al*., 2005).
Schaumburg *et al*., (2011) found 33% *S. aureus* nasal carriage among a remote Gabonese Babongo Pygmies. Another Gabonese study documented 29% nasal

colonization of *S. aureus* among hospital staff and inpatients (Ateba Ngoa *et al*., 2012). A study recently reported a nasal carriage of *S. aureus* between inpatients (14%) and staff (23%) at the largest hospital in Ghana with MRSA prevalence of 1.3% (Egyir *et al*., 2013). In another investigation, Egyir *et al*., (2014b) identified 21% *S. aureus* and 0.3% MRSA nasal carriage between two communities in Accra. A low prevalence of MRSA (3%) by molecular detection of *mec*A was also reported among *S. aureus* isolates collected from healthcare institutions across Ghana (Egyir *et al*., 2014a). In contrast, 33.6% MRSA prevalence was identified from all *S. aureus* isolates collected from five hospitals in Accra using the PBP2a slide latex agglutination test (Odonkor *et al.*, 2012). The difference in prevalence may be due to the different methods of identification of *S. aureus* and MRSA. It has been noted that, due to the relative insufficient number of laboratory facilities in Ghana, treatment of illness in Ghanaian healthcare centres is mainly empirical (Newman *et al*., 2011) and hence only few epidemiological data exists in Ghana.

Accurate typing methods are important for local and national *S. aureus* and MRSA surveillance, outbreak investigations and international comparisons. Studies have suggested that, to develop effective strategies to control the dissemination of *S. aureus*, an in-depth understanding of its spread and evolution is required. In view of this, several typing methods have been developed which are employed in tracking, identifying and understanding the epidemiology of *S. aureus* and MRSA within the hospital environment and the community. The choice of the typing method depends on the problem or the epidemiological situation (Mongkolrattanothai, 2013; Rodriguez *et al*., 2015).

1.2. PROBLEM STATEMENT

It is documented that, patients colonized or infected with *S. aureus* have been known to shed *S. aureus* resulting in contamination of their skin, clothing and environmental surfaces. There is also a risk of transmitting MRSA infections from person-to-person directly or indirectly via contaminated devices, hands of personnel, equipment or environmental surfaces (Sexton *et al*., 2006 ; Dietze *et al*., 2001). *S. aureus* and MRSA nasal colonization is associated with the development of subsequent infection both inside and outside of the hospitals (Butterly *et al*., 2010). Despite this well-established relationship between nasal carriage and infections, not much data is available in Ghana regarding the nasal carriage of *S. aureus* and MRSA among Children.

1.3. JUSTIFICATION

S. aureus especially MRSA is implicated in a variety of serious life threatening infections and its asymptomatic nasal carriage is identified as an important risk factor for both nosocomial and community acquired infections (Schaumburg *et al*., 2014 ; Wertheim *et al*., 2005). Nasal colonisation of MRSA among hospitalised patients is significant not only in terms of predisposing to subsequent infections, but also in playing an important role in transmission among hospital staff, to other patients and their family members (Wertheim *et al*., 2005). The presence of *S. aureus* does not necessarily mean infection but screening helps identify the potential sources from which this organism can be acquired or transmitted to other individuals. The emergence of resistance strains of *S. aureus* as well as the rising prevalence in the community highlights the need for worldwide epidemiological studies of this pathogen. Also, certain virulence factors are associated with distinct *S. aureus* diseases and hence the importance of examining the genes encoding virulence factors.

The importance of gaining better understanding of the epidemiology of nasal colonisation of *S. aureus* among hospitalised children locally using the appropriate typing methods is also highlighted by the increasing demand for better control of MRSA and establishing effective infection control measures. This research therefore seeks to contribute to our understanding of the epidemiological trend of virulence, antibiotic resistance and evolution of *S. aureus* for current and future epidemiological and clinical studies.

1.4. RESEARCH QUESTIONS

1. What is the prevalence of nasal carriage of *S. aureus* among hospitalized children in Ghana?
2. What is the current trend of antibiotic susceptibility pattern, virulence and genotypes of *S. aureus* among hospitalized children in Ghana?

1.5. AIM

The aim of this study was to describe the epidemiology of *S. aureus* and MRSA nasal carriage among hospitalized children at the Agogo Presbyterian hospital.

1.5.1. Specific objectives of this research

1. To determine the prevalence of nasal carriage of *S. aureus* and MRSA among hospitalised children.
2. To assess the antibiotic resistance profile of *S. aureus* among hospitalised children.
3. To determine the prevalence of Panton-Valentine leukocidin (PVL) toxin genes among *S. aureus* isolates from nasal carriage.
4. To investigate the diversity of *S. aureus* genotypes among hospitalised children.

CHAPTER TWO

LITERATURE REVIEW

2.1. MICROBIOLOGY OF *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus (*S. aureus*) as it is known in medical terms as *Staph aureus* is a bacterium that belongs to the Staphylococaceae family and the *Staphylococcus* genus. Members of this family are Gram-positive cocci, facultative anaerobic, non-spore forming and non-motile bacteria which appeared in grape-like or clusters (Foster, 1996). In 1881, Alexander Ogston identified these microscopically grape-like or clusters of bacteria from surgical abscesses in a knee joint, which was named *Staphylococcus* from the Greek word staphyle meaning "a bunch of grapes" (Ogston, 1882).

The identification of *S. aureus* is based on morphological and biochemical characteristics. All staphylococci consist of a Gram positive cell wall with a diameter of 0.7-1.2μm and are able to grow under aerobic and anaerobic conditions (facultative anaerobic). *S. aureus* produces β-hemolytic colonies when growing on media by producing hemolysins which include: α-toxin, γ-toxin, β-toxin, and δ- toxin (Foster, 1996). *S. aureus* are mostly golden-yellow to orange colonies on blood media due to the pigmentation from carotenoids; hence the species name *aureus*, meaning "golden". The carotenoids enhance the pathogenesis of *S. aureus* by inactivating the effect of superoxides and other reactive oxygen components within neutrophils (Liu, 2009). In contrast, the coagulase-negative staphylococci (CNS) such as *S. saprophyticus, S. epidermdis, S. haemolyticus* etc typically form relatively small grey-white colonies when growing on blood agar (Solati *et al.*, 2015). *Staphylococcus aureus* is differentiated from other members of the staphylococci family by producing the enzyme coagulase (Bannerman *et al.*, 2003). *S. aureus* produces free coagulase enzyme (staphylocoagulase)

and bound coagulase (clumping factor). Staphylocoagulase (free coagulase) is encoded by the coa gene and causes fibrinogen polymerization and clotting of plasma. The bound coagulase is encoded by the clfA gene and it converts fibrinogen to insoluble fibrin which causes the staphylococci to clump together. *S. aureus* can grow in a medium containing high salt of 7.5–10% of NaCl due to the production of osmoprotectants. It also produces yellow colonies on mannitol salt agar due to the fermentation of mannitol (Rogers *et al*., 2009). Organisms such as *Haemophilus influenza*, *Streptococcus pneumoniae* and *Staphylococcus aureus* thrive in the nasopharynx of healthy humans. Though colonisation with these organisms is harmless most of the time, it is believed to be responsible for the horizontal transmission at various population levels. Moreover, it increases the risk of endogenous infections (Peacock *et al*., 2003). *S. aueus* is often found as a normal microbiota of the skin, skin glands and mucous membranes but it is predominantly found in the nose of healthy person (van Belkum *et al*., 2009). According to Chamber and Deleo, (2009), the most important pathogen of the genus *Staphylococcus* that is capable of causing complicated infections in humans is *S. aureus*. Pathogenic strains produce a wide range of virulence factors that are associated with specific clinical conditions (Gordon and Lowy, 2008).

S. aureus genome comprises a circular chromosome which size ranges around 2.7 to 2.9 Mbp containing core and accessory genes. The core genome which is controls metabolism and housekeeping functions make up about 75% of the genome and are highly conserved. The rest are accessory genes which are not associated with essential functions for growth and survival and they include various virulence genes (Holden *et al*., 2004).

The rapid development of resistance to antibiotic in *S. aureus* especially methicillin resistance has contributed to its emergence as an important pathogen in clinical setting and in the community (Olofsson and Cars, 2007).

2.2. VIRULENCE FACTORS AND PATHOGENESIS OF *S. AUREUS*

Staphylococcus aureus pathogenesis is multifactorial that are directly or indirectly involved in causing infections. According to Gordon and Lowy, (2008), the pathogenesis of *S. aureus* is due to the ability of the organism to produce a wide range of virulence factors. These virulence factors include enterotoxins, exfoliative toxins, toxic shock syndrome toxin-1 (TSST-1), Panton-Valentine leukocidin (PVL), hemolysins, protein A and other enzymes. Infection may occur when the host defence systems are destroyed whether by shaving, surgery, injections or insertion of an indwelling catheter thereby providing the organism the opportunity to be inoculated into the sterile tissues from the sites of carriage (Boucher *et al*., 2010). These virulence factors are found on a chromosome, bacteriophages, transposons or plamids and the expression of multiple virulence factors is a key to its pathogenesis (Baba *et al*., 2008).

S. aureus has various surface proteins called 'microbial surface components recognizing adhesive matrix molecules' (MSCRAMMs) that mediate adherence to host tissues during infection. The protein A is one of the MSCRAMM and it binds to the IgG Fc-domain section of immunoglobulin and prevents opsonization. *S. aureus* is able to produce chemotaxis inhibitory proteins or extracellular adherence proteins, which interfere with chemotaxis to the site of infection and neutrophil extravasations. *S. aureus* also produces a necrotizing toxin called Panton Valentine leukocidin (PVL) that forms pores in the cell membrane of leukocyte causing their destruction (Gordon and Lowy, 2008). Another important step in *S. aureus* colonization as well as in its pathogenesis is the ability of this

organism to adhere to the host tissues. Proteins such as coagulase (free and bound coagulase), fibronectin-binding proteins, protein A and collagen-binding proteins can adhere to the extracellular matrix components of the host. Adherence to host tissues or other materials enables *S. aureus* to persist and grows in various ways (Foster and Hook,1998; Joh *et al*., 1999).

During *S. aureus* infection, it also produces various enzymes such as lipases, proteases and lactases which support it to infect and destroy host tissues and metastasize to other sterilize sites (Gordon and Lowy, 2008). *S. aureus* pathogenic factors are illustrated in Figure 2.1.

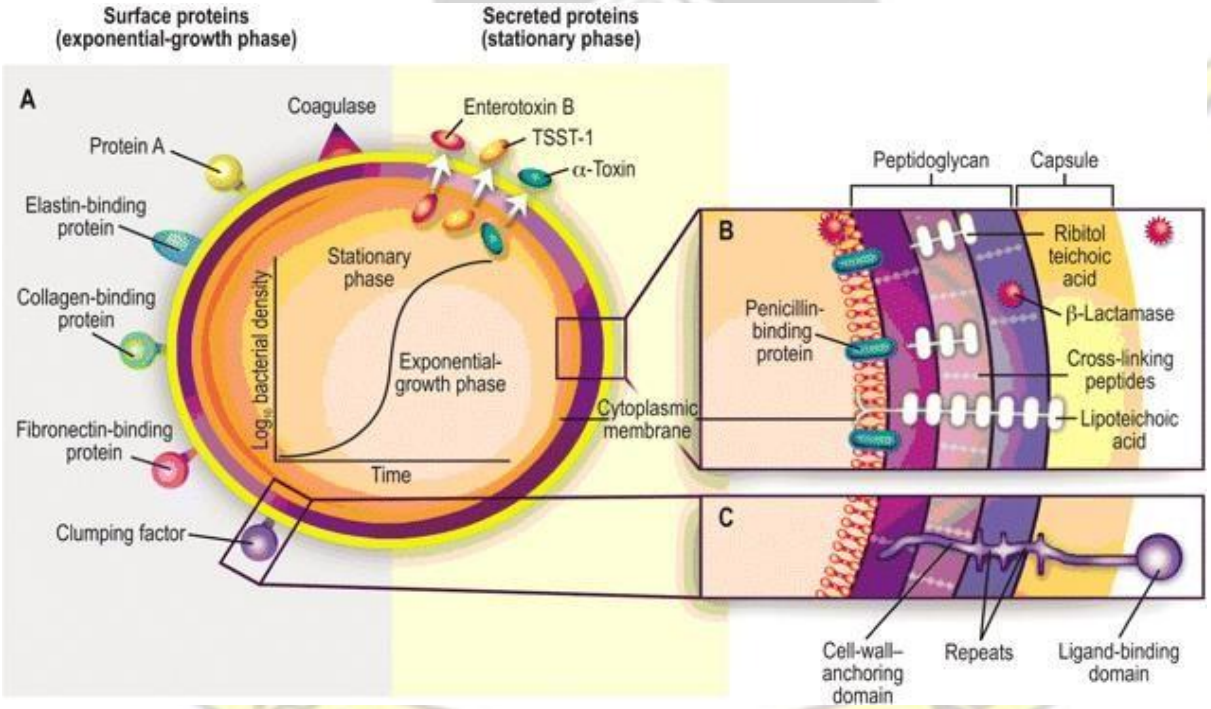

Figure 2.1: ***Staphylococcus aureus* pathogenic factors (Gordon and Lowy, 2008)**

2.3. *S. AUREUS* INFECTIONS

S. aureus causes a wide variety of infections ranging from relatively simple skin infections to serious life threatening invasive diseases in humans and animals (Nagel *et*

al., 2013; David and Daum, 2010). The skin and subcutaneous infections include: folliculitis, cellulitis, furunculosis, bulbous and impetigo and the systemic infections include: sepsis, wound, septic arthritis, pneumonia, endocarditis, abscesses, meningitis, osteomyelitis, as well as toxinoses with food poisoning, scalded skin syndrome (SSS) and toxic shock syndrome (TSS) (Shittu and Lin, 2007; Chamber and Deleo, 2009).

The response to *Staphylococcal* infection is usually inflammation of the localised area which is characterized by pus accumulation, tissue necrosis and swelling due to response by the immune system. *S. aureus* is capable of causing infections in the kidney which can lead to kidney failure, invade the heart or bloodstream causing endocarditis and infect meninges which can lead to meningitis (Gordon and Lowy,
2008). The production of toxins by certain *S. aureus* strains causes toxemic syndromes. These syndromes include staphylococcal scalded-skin syndrome (SSSS) which is due to exfoliatins and staphylococcal food poisoning which is due to staphylococcal enterotoxins (SEs). Others include toxic shock syndrome (TSS) and staphylococcal scarlet fever which is due to toxic shock syndrome toxin 1 (TSST-1) and SEs, (Shittu *et al*., 2007). In fact, anyone can get a *staph aureus* infection especially MRSA but there are certain groups of people who are at a greater risk and these include people with health conditions such as diabetes, lung disease, vascular disease, cancer and eczema (Wertheim *et al*., 2005; Von Eiff *et al*. 2001). The risk of acquiring more serious staph infections is higher in a healthcare setting because patients often have weakened immune systems or have undergone procedures such as surgery or have intravenous
catheters.

Boucher and Corey, (2008) estimated that, about 19,000 hospitalized American patients died annually as a result of MRSA. Recent analyses have also identified *S. aureus* as the main etiological agent of many bacterial infections in sub-Saharan Africa (Shittu *et al*., 2011; Schaumburg *et al*., 2014). There is an increased morbidity, mortality and cost associated with MRSA infections. This cost comes either in the form of expenses due to prolonged hospital stay, the loss of productivity, additional diagnostic and antibiotic use or long term disability (Grundmann, 2006; Abdulager *et al*., 2015).

2.4. NASAL CARRIAGE AND RISK OF *STAPHYLOCOCCUS AUREUS* INFECTION

S. aureus colonizes the skin and mucosae regions of human beings and other animal species with the nose being the primary ecological niche (Wertheim *et al*., 2005). The nose is composed of keratinised epithelial tissue, large apocrine and sweat glands and this plays a key role in *S. aureus* adhesion (Bibel *et al*., 1982). Carriage of *S. aureus* in the nasal cavity is usually asymptomatic but this carriage usually increases the risk of developing subsequent *S. aureus* infections when the skin and mucous barriers are breached or if the immune system is compromised (Chamber and Deleo, 2009).

According to van Belkum *et al.*, (2009), approximately 20-30% of the human population is colonized persistently with *S. aureus* in the nose and 30% are transiently colonized during their lives. The rate of acquiring MRSA infection in children is similar to that seen in adults. However, the risk of developing subsequent infection in children and adult varies depending on situations. *S. aureus* carriage is also more frequent among patients with diabetes and HIV and younger children (Milstone *et al*., 2011). Nasal carriage of *S. aureus* is also associated with an increased risk of *S. aureus* infection among inpatients receiving ambulatory peritoneal dialysis, in patients after surgery and in patients receiving haemodialysis (Kluytmans *et al*., 1997; von Eiff *et al*., 2001). In the study by

Von Eiff *et al*., (2001), 14 patients originally colonized with *S aureus* developed *S aureus* bacteraemia later and 12 (86%) of these patients had *S. aureus* strains identical to those obtained from the blood. Similar results have been reported by Wertheim *et al* (2005) with 32 of the 40 (80%) invasive *S aureus* strains being identical to the nasal colonisation strains detected. Colonized patients serve as the chief source of *S. aureus* in healthcare settings. In 1931, Danbolt who studied furunculosis first reported the association between nasal carriage of *S. aureus* and staphylococcal diseases (Klutymans *et al*., 1997). Data on nasal carriage in children, appears to be somewhat similar to the prevalence reported in adults, ranging from 18.12 to 38.5%, but there are a wide variability between results from different countries and different studies (Lucia Preoţescu and Streinu-Cercel, 2013). Nasal carriage studies have been carried out among various populations in Ghana with varying results from

13.9% to 23% (Egyir *et al*., 2013; Egyir *et al*., 2014b).

2.5. EPIDEMIOLOGY OF *S. AUREUS* AND MRSA

MRSA is currently accounting for 20–60% of all *S. aureus* infections in many countries and has become a great burden in public health (Grundmann *et al*., 2006). According to studies review by Falagas *et al*., (2013), the prevalence of MRSA was either between 25% and 50%, or less than 25%. In Asian countries, MRSA accounts for about 67.4% of hospital associated (HA) or nosocomial *S. aureus* infections and 25.5% of community associated *S. aureus* (CA-SA) infections (Song *et al*., 2011). The prevalence of MRSA varies greatly with the studied population, type of hospital and geographical location. However, high prevalence has been reported in hospitals in European countries, Asia, US and South America (Diekema *et al.*, 2001).

A recent review in Africa indicated that the prevalence of MRSA appears to be increasing in many African countries since the year 2000 and this poses a serious threat to public health (Falagas *et al*., 2013). Of the 200 children age 5- to 7-year-old screened by Oguzkaya-Artan *et al*., (2008), 36 (18%) and 2 (5.6%) *S. aureus* and MRSA were isolated respectively. A Gabonese study found 29% *S. aureus* and 3% MRSA nasal colonization among hospital staff and inpatient (Ateba Ngoa *et al*., 2012). Studies in Ghana have also reported similar rates of *S. aureus* and MRSA nasal carriage. Egyir *et al*., (2013) reported 14% *S. aureus* among inpatients and 1.3% MRSA among all the participants with 10% prevalence among the isolates from the inpatients. Another study reported 0.3% MRSA and an overall prevalence of 21% *S. aureus* nasal carriage in Accra (Egyir *et al*., 2014b). High prevalence of MRSA and *S. aureus* has been reported from studies in Ghana (Odonkor *et al*., 2012; Saana *et al*., 2013; Tagoe *et al*., 2011).

Despite the limited molecular epidemiological data in Africa, the few studies have identified some common strains. These predominant strains among isolates include t355 (ST152) and t084 (ST15). These strains have also been reported to be common among *S. aureus* isolates from Ghana (Egyir *et al*., 2014a; Breurec *et al*., 2011), suggesting that these strains are predominantly found in the human population in Ghana and are wide spread in Africa.

2.6. *STAPHYLOCOCCUS AUREUS* TRANSMISSION AND INFECTIONS CONTROL

The MRSA is associated with high rate of morbidity and mortality due to treatment failure. Increased costs due to durations of hospitalization and use of more-expensive antimicrobial agents in the treatment of MRSA is widely acknowledged (Abdulager *et al*., 2015). Proper cleaning and disinfection of environmental surfaces, equipment, beds and frequently touched surfaces are proposed for controlling *S. aureus*. Also,

environmental hygiene, hand washing with water or antiseptic soap and the use of disinfectants have also been cited as important practices to reduce the transmission of infectious in healthcare settings (Siegel *et al*., 2007). Miller and Diep, (2008) advocated active surveillance, isolation of carriers and general preventive practices such as hand hygiene, decolonization and standard precautions. More importantly, training of health care workers and sensitization of the general public on antibiotic stewardship are also very important aspects of controlling the spread of infections due to *S. aureus* (Newman *et al*., 2011).

2.7. MECHANISMS OF ANTIBIOTIC RESISTANCE IN BACTERIA POPULATION

Bacteria may have innate resistance or acquired resistance to antimicrobial agents. This involves; mutations in cell genes (chromosomal mutation) leading to resistance, transformational gene transfer from one microorganism to another, conjugation which needs independent genetic elements including transposons (Tns) and plasmids and also transduction which involves independently replicating bacterial viruses, known as bacteriophages (Giedraitiene *et al*., 2011). The current class of antimicrobial agents targeted certain major bacterial processes such as metabolic pathways, cell wall, functions of the cell membrane, protein and nucleic acid synthesis (Tenover, 2006). After the bacteria gain resistance genes against various antimicrobial agents, the bacteria can therefore use several biochemical types of resistance mechanisms including drug inactivation or enzymatic degradation involving enzymes that destroy the antibiotic. These enzymes include B-lactamases that cleave the amide bond in the B-lactam ring (Tomanicek *et al*., 2011). Another mechanism is by extrusion of the antibiotic before it reaches the target by efflux pump. This mechanism extrudes the antibiotic before it

reaches the bacteria target-site (Tenover, 2006). Target modification occurs by an altered bacteria cell wall which modifies the target region. An example is

MRSA which involves a modified Penicillin Binding Protein (PBP2a) that confers methicillin resistance to certain *S. aureus* strains (Tomanicek *et al*., 2011).

2.8. ANTIMICROBIAL CHEMOTHERAPY AND *S. AUREUS*

Antibiotics or antimicrobial agents are substances used to kill or stop micro-organisms from growing. It is however noted that, the introduction of new antibiotics to treat *S. aureus* infections has frequently been followed by the emergence of resistant strains (Schito, 2006). *S. aureus* is particularly adaptable at acquiring mechanisms of resistance to clinically relevant antibiotics and the emergence of multiresistant strains possesses a serious therapeutic challenge and economic burden (Newman *et al*., 2011).

2.8.1. β-lactam Antibiotics

This class of antibiotics comprises a group of bacterial cell wall synthesis inhibitors.

The most clinically relevant families of β-lactam antimicrobial agents include the penicillins (penams), carbapenems (penems) and cephalosporins (cephems) (Prescott, 2013). These antimicrobial agents have a bactericidal effect on *S. aureus* as they express the penicillin-binding proteins (PBPs) responsible for transpeptidation of the peptidoglycan layer, thereby disrupting cell wall integrity (Tenover, 2006).

Penicillins

In the early 1940's, β - lactam antibiotics such as penicillin was introduce in clinical use and invasive *S. aureus* infections experienced responsiveness to treatment for the first time with decreased in mortality from *S. aureus* infections (Rice, 2012). *S. aureus* became resistance to penicillin only a year after its introduction and currently, 80%– 95% of strains of *S. aureus* throughout the world are penicillin- resistant (Sakoulas and

Moellering, 2008). Penicillin-resistance is due to the production of a β-lactamase (penicillinase). This enzyme inactivates β-lactam antibiotics by the hydrolysis of the βlactam ring that is essential to the β-lactams antimicrobial activity. The *blaZ* that encodes β-lactamase in *S. aureus* is carried by a transposable element located on a plasmid. This often contains genes encoding resistance to other antibiotics (Jensen and Lyon, 2009). **β- lactamase- stable β - lactam agents**

Antibiotics such as methicillin, oxacillin, cloxacillin and flucloxacillin were introduced as antistaphylococal agents to fight *S. aureus* that are resistant to penicillin because they are β-lactamase inhibitors (Jensen and Lyon, 2009). Methicillin-resistant *S. aureus* (MRSA) was first discovered in 1961 just after the introduction of methicillin (Jevons, 1961).

Mechanism of Methicillin Resistance

The acquisition of the *mecA* gene which encodes a unique penicillin binding protein (PBP2a) results in resistance to methicillin. This unique PBP2a has a reduced affinity for β-lactams. This PBP2a causes resistance to all β-lactam antibiotics as it inhibits binding at the active site for β-lactams. This *mecA* is carried by a large mobile heterogeneous staphylococcal chromosome cassette *mec* (SCC*mec*) element (Prescott, 2013).

2.8.2. Macrolides, Lincosamides & Streptogramins

Macrolides, lincosamides and streptogramins (MLS) were first introduced in 1952. Their target site is the bacterial 50S ribosomal subunit which they inhibit protein synthesis (Schito, 2006). Examples of macrolides include: erythromycin, roxitromycin, clarithromycin, spiramycin and josamycin. Treatment of diseases with lincosamides such as clindamycin, lincomycin and pirlimycin was limited because of the rapid development of resistance and their side-effects (Morar *et al*., 2009).

Mechanism of resistance

According to Jensen and Lyon, (2009), resistance to the MLS group of antibiotics is primarily due to the *ermA*, *ermB* or *ermC* genes which are located on plasmids or chromosomes. Their effect is a reduction in the affinity of the target site by the antibiotic, by active extrusion of the antibiotic out of the cell or by enzymatic modification of the antibiotic.

2.8.3. Fluoroquinolones

Fluoroquinolones are an important class of broad spectrum antibacterial agents**.** The primary targets of quinolones are bacterial DNA gyrase subunits (gyr1 and gyrB) and topoisomerase IV which are involved in DNA replication. The quinolones are divided into two major subgroups which include those with fluorine substituent (ciprofloxacin, ofloxacin and pefloxacin) and those without fluorine substituent on their ring (pipemidic and oxolinic acids) (Dougherty *et al*., 2001).

Mechanism of resistance

Resistance to quinolone occurs as a result of spontaneous chromosomal mutations in the quinolone resistance-determining region. They inhibit nucleic acid synthesis by targeting topoisomerase IV and DNA gyrase (Sierra *et al*., 2005). Resistance to quinolones in *S. aureus* usually arises by efflux pump and the acquisition of chromosomal mutations in the *gyrA* and *gyrB* genes that alters the drug target region

(Fabrega *et al*., 2009)

2.8.4. Aminoglycosides

Aminoglycosides comprise protein synthesis inhibitors that have a bactericidal effect mediated by binding with the 30S ribosomal subunit thereby disrupting the translocation

of peptidyl-tRNA. Drugs belonging to this antibiotic group are gentamycin, tobramycin, netilmicin and amikacin with similar activity to streptomycin but with reduced toxicity (Durante-Mangoni *et al*., 2009).

Mechanism of Resistance

Resistance to these antibiotics is usually enzymatic due to modification of the antibiotics by the bacteria. Modified aminoglycosides bacteria possess the aminoglycoside-modifying genes *acc, aph* and *ant* which make the antibiotics unable to bind with the ribosomes and therefore no longer able to inhibit bacterial protein synthesis (Schito, 2006; Jensen and Lyon, 2009). However, resistance can also be due to mutations that reduce the uptake of aminoglycoside (Durante-Mangoni *et al*., 2009).

2.8.5. Tetracyclines

Tetracyclines (tetracycline, doxycycline, minocycline, oxtetracycline) are antibiotics which exhibit bacteriostatic and bactericidal activities. It has been noted that, tetracyclines are not the treatment options for *Staphylococcus aureus* infections due to the wide resistance developed by most strains of *S. aureus* to this group of antibiotics (Rayner and Munckhof, 2005).

Mechanism of resistance

Resistance to tetracycline identified in species of *Staphylococcus aureus* involves two mechanisms: (i) active efflux mediated by the *tet*K and *tet*L genes, (ii) ribosomal protection due to the *tet*M or *tet*O determinants that are located either on a chromosome or transposon. Tetracycline resistance is usually associated with a decrease in the intracellular accumulation of the antibiotic in *S. aureus* and it was originally associated with reduced uptake of the antibiotics (Jensen and Lyon, 2009)

2.8.6. Glycopeptides

Vancomycin and teicoplanin are the glycopeptides mostly used as the mainstay of treatment of serious infections caused by MRSA (Jensen and Lyon, 2009). The glycopeptides exert their bactericidal effect by preventing cell-wall synthesis due to the binding of the D-alanyl-D-alanine C-terminus of the peptidoglycan precursors of the bacteria (Sakoulas and Moellering, 2008). However, Vancomycin-intermediate *S. aureus* (VISA), heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and Vancomycin-resistant *S. aureus* (VRSA) have been detected among *S. aureus* isolates (Schito, 2006).

Mechanism of resistance

The presence of the *van* operon in VRSA results in the production of peptidoglycan precursors with the D-alanyl-D-lactate terminal residues, which have a low-affinity for vancomycin compared to D-alanyl-D-alanine residues; therefore cell wall synthesis is unaffected by the presence of this antimicrobial agent (Sakoulas and Moellering, 2008).

2.8.7. New classes of antimicrobial agents

A number of new antimicrobial agents against resistant *S. aureus* strains, including VISA and VRSA strains have recently been introduced into clinical practice, which includes linezolid, daptomycin and tigecycline. These broad spectrum antibiotics act against gram positive bacteria including MRSA (Rayner and Munchkof, 2005).

2.9. *S. AUREUS* RESISTANCE TO ANTIMICROBIAL AGENTS IN GHANA

Antimicrobial drugs have been proven useful for treating bacterial infections but the emergence of antimicrobial drugs resistant strains have been observed in almost all pathogenic bacteria populations (Newman *et al*., 2006). Most reports have indicated that bacteria have developed resistance to commonly and relatively cheap antibiotics which have become the main treatment options for decades. Antibiotic resistance has been

attributed to the misuse of antibiotics, which exerts selective pressure favouring the emergence of resistant strains (Alanis, 2005; Newman *et al*., 2011). The prevalence of antibiotic resistant strains of *S. aureus* varies between different countries. In Ghana, resistance to commonly use antibiotics have been reported in hospitals (Newman *et al*., 2011).

Donkor *et al*., (2013) documented *S. aureus* resistance rates >40% for the various antibiotics used in children with and without HbSS disease with the exception of erythromycin and cloxacillin. Egyir *et al*., (2014a) also reported that except for penicillin (97%), tetracycline (42%) and erythromycin (6%) antimicrobial resistance was below 5% for all antibitiocs tested. Odonkor *et al.*, (2012) also recorded 33.6% of *S. aureus* isolates from Accra to be oxacillin resistance and were considered MRSA strains. These MRSA strains were susceptible to erythromycin (75%), gentamicin (54.7%), cotrimoxazole (49%), cefuroxime (38%), flucloxacillin (28.6%), ampicillin (15.5%) with Penicillin (4.8%) and tetracycline (7.1%) been the least susceptible. There is a high resistance to penicillin and tetracycline of *S. aureus* strains in Ghana and this is similar to what has been reported in other Africa countries (Shittu *et al*., 2011; Egyir *et al*., 2014a).

2.10. EVOLUTION OF MRSA

Methicillin was introduced in clinical settings after there was wide spread of penicillin resistance and this was rapidly followed by reports of MRSA strains that were resistant to almost all β-lactam antibiotics. Today, MRSA strains are found worldwide (Song *et al*., 2011) . The primary mechanism by which *S. aureus* becomes resistant to methicillin is by the acquisition of *mec*A gene which encodes the acquired penicillinbinding protein (PBP2a). This *mec*A gene is carried on a mobile genetic element called staphylococcal

cassette chromosome *mec* (SCC*mec*). There are 5 SCC*mec* subtypes (types I–V). The PBP2a has a low affinity for β-lactams and is capable of substituting the biosynthetic functions of the normal PBPs even in the presence of B-lactams antibiotics, thereby preventing cell lyses (Mathews *et al*., 2010; Deurenberg and Stobberingh, 2008). Historically, MRSA are noted to be hospital-acquired or associated with nosocomial infections and are usually resistant to multiple drug classes. These hospital-acquired/associated MRSA (HA-MRSA) possess SCC*mec* types I, II, or III. However, different strains with unique characteristics have emerged among people in the community without the known risk factors for HA-MRSA**.** These strains, termed Community-associated/acquired MRSA (CA-MRSA) emerged since 1990. These strains are usually susceptible to other antimicrobial classes but are resistant to βlactams. Types IV and V are associated with CA-MRSA isolates and frequently carries PVL (David and Daum, 2010; Van Belkum *et al.*, 2009).

2.11. DIAGNOSIS AND LABORATORY IDENTIFICATION OF *S. AUREUS*

The diagnosis of *S. aureus* infections depends on the type of infections or symptoms of the patient and also the healthcare personnel evaluation. The appropriate sample is collected after the evaluation. These samples are sent to the laboratory for screening and identification using biochemical or molecular tests (Bannerman *et al*., 2003). In the laboratory, a Gram stain can be performed and observed under the microscope.

Suspected specimen is cultured on blood agar or selective medium such as Mannitol Salt Agar (MSA) or other chromogenic agars. Typical colonies of *S. aureus* should show a big creamy yellow on blood agar or surrounded by yellow area on MSA. These characteristic colonies are selected for further analysis. Differentiation at the species level is by biochemical tests such as catalase test and coagulase test (Kloos and

Schleifer, 1975). A typical *S. aureus* are catalase and coagulase positive. Recently, molecular based methods are currently used for the confirmation of isolates of *S. aureus*. The detection of *nuc*A gene by PCR is mostly used to confirm isolate as *S. aureus* (Hamdan-Partida *et al*., 2010; Brakstad *et al*., 1992). However, enzyme based assays such as enzyme-linked immunosorbent assay (ELISA) and antigen tests are also good methods for diagnosis. In fact, different infections or symptoms of *S. aureus* require various combinations of currently used diagnostic methods.

2.12. TREATMENT OPTIONS FOR *S. AUREUS* INFECTIONS

Historically, the treatment option for *S. aureus* infection was penicillin which was derived from *Penicillum fungus*. However, due to the high resistance to penicillin in many countries, penicillinase-resistant antimicrobials such as oxacillin or methicillin were developed to treat *S. aureus* infections (Sakoulas and Moellering, 2008). Combination with gentamicin may also be used to treat serious infections like endocarditis but it has a high risk of damage to the kidney (Cosgrove *et al*., 2009). Methicillin resistant *S. aureus* (MRSA) have acquired resistant to most of these antibeta-lactamase antimicrobials (Gorwitz *et al*., 2008). Currently, the treatment option for severe invasive MRSA infections is the glycopeptides, such as vancomycin but there are reports of glycopeptides resistance (Schito, 2006). Newer classes of drugs, such as linezolid are still effective against HA-MRSA and CA-MRSA, but there is a reduction in the sensitivity in recent years (Rayner and Munchkof, 2005).

2.13. EPIDEMIOLOGICAL TYPING METHODS FOR *S. AUREUS*

The use of accurate typing methods is important for monitoring the geographic distribution of bacterial clones in hospitals and in the communities (Robinson and Enright, 2004). Epidemiological typing techniques widely used for characterising *S.*

aureus isolates include Staphylococcal protein A (*spa*) typing, polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (Deurenberg and Stobberingh, 2008). MLST and *spa* typing are sequenced based typing methods, which are mostly used for typing *S. aureus*. The sequence-based typing methods produce portable and unambiguous data leading to the creation of central databases, which enables the comparison of local data obtained with previous studies in different geographical locations (Rodriguez *et al*., 2015). Typing allows the detection of transmission events and patterns, from within a single healthcare facility, between continents and therefore important in infection control and public health microbiology. Secondly, virulence and resistance properties of specific isolates can be inferred from typing and so can directly influence clinical decisions. Finally, typing reveals the population structure and provides insight into the general principles of the species' natural history (Strommenger *et al*., 2008). A brief overview of some of the most widely used typing methods is given below.

2.13.1. Antibiogram

Isolates of *Staphylococcus aureus* can be compared based on their susceptibility to the available antibiotics. This antibiotic susceptibility is easy to perform, is cheap, gives rapid results and above all, it is available in most microbiological laboratory. The disadvantages of this method however, include poor discriminatory ability and lack of reproducibility (Montesinos *et al*., 2002). The antibiotic resistance pattern of a particular organism is influenced by the local environment and also, unrelated strains may produce the same antibiogram as a result of similar selective pressure upon them. Categorization of bacteria into susceptible and resistant based on antibiotic susceptibility cannot differentiate between unrelated strains in practice. Also the antimicrobial susceptibility

pattern of an individual strain may change during treatment or because of antibiotic selection pressure in the hospital (Joshi, 2010).

2.13.2. Pulsed Field Gel electrophoresis (PFGE)

This typing method is accepted as the "gold standard" method for typing several bacteria species. PFGE is the most common method use for studying local or shortterm epidemiological studies of *S. aureus* (Narukawa *et al*., 2009). In performing PFGE for *S. aureus*, the chromosomal DNA is digested with the restriction enzyme *SmaI* and the DNA fragments or products are analysed by agarose gel electrophoresis in an electric field with an alternating voltage gradient. The band patterns are analyzed using a special software package, such as Gel Compar II from Applied Maths, Dice comparison and unweighted pair group matching analysis (UPGMA) settings (Tenover *et al*., 1995). In PFGE analysis, the *smaI*- restricted *S. aureus* genomes obtained are compared to determine their genetic relatedness and also compared against reference genotypes (Shukla *et al*., 2012). This method has proven very successful in providing genetic diversity of *S. aureus* and MRSA clones that have the ability to spread internationally and cause major outbreaks (epidermic MRSA clones; EMRSA). However, reproducibility and inter-laboratory reliability are its limitations (Lamers *et al*., 2011).

2.13.3. Polymerase Chain Reaction (PCR)

This method is used to amplify short regions of DNA in vitro. In this particular method, the target DNA is amplified by a thermostable DNA polymerase enzyme, in the presence of oligonucleotide primers and nucleotides. During, the multiple cycles of denaturation and extension in the thermocycler, it leads to exponential amplification of the target DNA. This exponentially amplification generates billions copies of the target DNA through denaturation, primer hybridisation and primer extension (Yang and Rothman, 2004). The PCR process takes place in a thermocycler and consists of three major steps:

(1) denaturation (2) primers annealing and (3) extension of primers (Yang and Rothman, 2004). The denaturation is the first step and takes place at high temperatures separating the double strand DNA into two single strands. The next step is annealing which involves annealing of the primers to their complementary region of the template DNA. The significance of the annealing temperature is that it determines the specificity (stringency) of the reaction. The last stage is the DNA synthesis or extension by which a thermostable DNA polymerase enzyme (Taq DNA polymerase), extends the primers. The Taq DNA polymerase works in the presence of deoxyribonucleotide triphosphates (dNTPs) and Mg2+ions (Trindade *et al*., 2003). The schematic

illustration of the PCR procedure is shown in Figure 2.3.

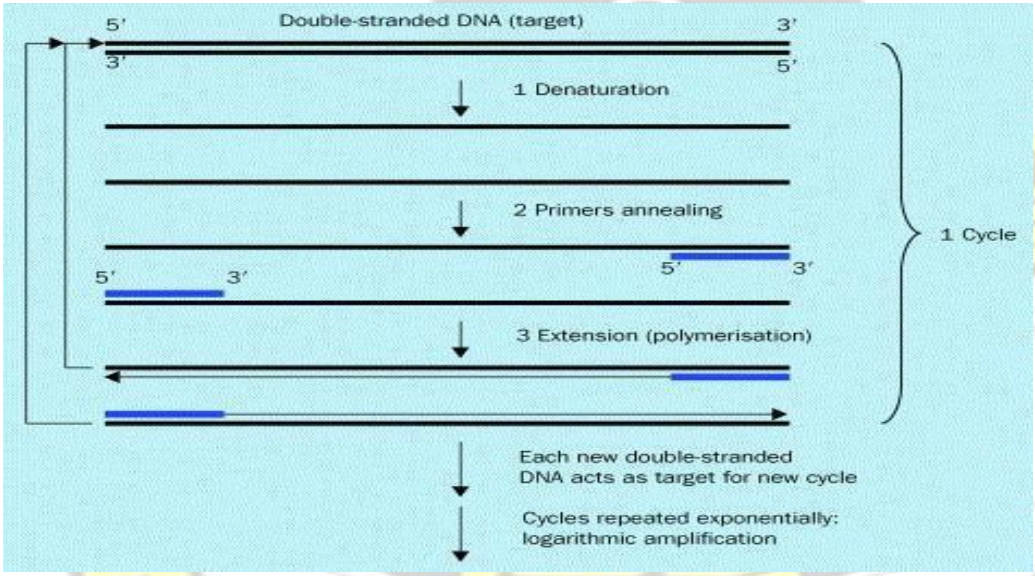

Figure 2.2: The schematic illustration of the PCR procedure

Multiplex PCR involves the incorporation of multiple sets of primers in a single reaction vessel which enables the detection of several DNA target sequences simultaneously. Real-time PCR simultaneously amplifies and detects the amplified products. The amplified products in real time PCR are measured as they develop by fluorescence resonance emission transfer probes, TaqMan probes or molecular beacons

((Pusterla *et al*., 2006).

2.13.4. Nucleotide sequencing

DNA sequencing techniques are key tools for promoting new discoveries. The Sanger sequencing method that has widely been used in many research fields was developed by Fred Sanger. It is now further improved since its discovery and is available in a semi-automated version. The Sanger sequencing resembles the ordinary PCR method and employs a DNA polymerase, dNTPs and primers, among other essential components. It is performed in a cyclic program. However, the DNA synthesis is performed using only one oligonucleotide primers in each tube to sequence each strand separately. The sequencing is performed in repeated cycles that will generate a mixture of DNA sequences differing in size and each terminal ddNTP is labelled with four different fluorescent colours. The products of the cyclic sequencing are subsequently separated by an automated capillary electrophoresis-based genetic analyser. The sizeseparated and fluorescently labelled DNA fragments will move through a laser that will emit the specific dye, which is detected by an optical device. All the four bases are detected by the labelled colours that emit light at different wavelengths. A laser then detects the specific colour of each DNA fragment and the sequence is shown as an electropherogram (Sanger *et al*., 1997).

2.13.5. Staphylococcal Protein A (spa) Typing

Another typing method is *spa* typing which evaluates the variation in the sequence in the polymorphic X-region of the *spa* gene, which encodes staphylococcal protein A (Strommenger *et al*., 2008). The *spa* locus consists of 24-bp repeats and its diversity is attributed to deletions, duplications of the repeats and point mutations. The *spa* typing is a sequence-based approach that is used to characterize *S. aureus* and MRSA. It has high portability, discrimination and ease of use. It is rapid and enables the reliable allocation

of isolates base on their lineages **(**Strommenger *et al*., 2008)**.** The advantage of *spa* typing over the other sequence base typing methods is that, it is simple, since it involves sequencing of only a single locus. It is therefore less laborious, less time consuming and less expensive (Deurenberg and Stobberingh, 2008).

The *spa* typing can be used to study both outbreaks as well as the molecular evolution of *S. aureus* and MRSA due to its relatively simple nature (Narukawa *et al*., 2009).

Another advantage of *spa* typing is that, the software package StaphType (Ridom GmbH, Würzburg, Germany) is available and is most widely used for the analysis of *spa* sequences in Europe. It is also used in reference laboratories worldwide to analyze the *spa* sequence chromatograms. There is a *spa* database to which *spa* typing data are synchronized and is available at http://spaserver.ridom.de and the *spa* server database is one of the largest typing databases for *S. aureus* (Deurenberg and Stobberingh, 2008).

According to Mellmann *et al*., (2007), the implementation of the clustering algorithm Based Upon Repeat Patterns (BURP) into Staph type makes cluster analysis based on *spa* typing (*spa* clonal complexes) possible.

2.13.6. Multilocus Sequence Typing (MLST)

MLST is a sequenced based typing method by which *S. aureus* strains are analysed for their evolution and lineages. MLST is a highly discriminatory typing method use for the characterization of bacterial isolates based on the sequences of the internal fragments of the seven housekeeping genes i.e. *arcC, aroE, glpF, gmk, pta, tpi* and *yqiL,* each approximately 500-bp in length. Each of these housekeeping genes is sequenced, analysed and assigned a distinct allele. The combination of these allele profiles of the seven genes define the *S. aureus* lineage and is designated sequence type (ST). The strain

ST247 has MLST allele profile 3-3-1-12-4-4-16 (www.mlst.net). The algorithm based upon related sequence types (BURST) defines the clonal complexes (CCs) and projects the evolutionary events within a *S. aureus* population. The ST are grouped within a single CC with the ST with the largest number of single locus variants (SLV) been the ancestor of a CC or when 5 of the 7 housekeeping genes have identical alleles profile. Several *spa* types belong to a single sequence type (ST) as determined with MLST, but they remain within an assigned clonal cluster. The data obtained by MLST can provide the evolutionary and population biology of bacterial species (Enright *et al*., 2000). The MLST website for *S. aureus* (http://www.mlst.net) is currently used for the epidemiological study of this species and the surveillance of hypervirulent and antibiotic-resistant clones. The disadvantage of this method is that it is laborious, expensive and time consuming (Cookson *et al*., 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1. STUDY DESIGN

This study was a hospital based cross-sectional study on the nasal carriage of *S. aureus* among hospitalized children at the Agogo Presbyterian Hospital (APH) in the Ashanti Akim North Municipality (AANM) from April, 2014 and January, 2015. The study was conducted at the children's ward at Agogo Presbyterian Hospital with culture, isolation and PCR done at the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) and *spa* typing was done at the Bernhard-Nocht Institute for Tropical Medicine (BNITM) and University of Bonn.

3.2. STUDY AREA

This research was conducted in Ashanti Akim North Municipality. It is one of the 27 districts in the Ashanti region of Ghana. The study area occupies a land area of about 1,160 square kilometres. The study area is located in the eastern part of Ashanti Region. It is situated in a tropical rain forest with temperature range between 20^{o}C and 36^{o}C. The Ashanti Region is the third largest region in Ghana and occupies approximately 10.2% of the total land area of Ghana. The 2010 population and housing census estimated the population of the Asante Akim North Municipality to be approximately 140,694 and it is suggested that about 40% of the total population are <15 years of age. Majority of the people are engaged in subsistence farming, animal husbandry and forestry. However, significant number of the people is also engaged in trading (www.asanteakimnorth.ghanadistricts.gov.gh).

3.3. STUDY SITE

This research was conducted in the paediatric ward of the Agogo Presbyterian Hospital. This hospital was established on 21st March, 1931 and is situated at Agogo in the

Asante Akim North district of the Ashanti region. It is a district hospital that serves the Asante Akim North Municipality and other catchment areas. The hospital has a bed capacity of 330 with a staff population of 400. It is the second largest hospital in the Ashanti Region after Komfo Anokye Teaching Hospital (APH, 2014). The hospital provides specialized care in ophthalmology, paediatrics, obstetrics/gynaecology, general surgery in addition to the general medical care and this attracts patients from all parts of the country as well as other countries such as Togo, Burkina Faso and Cote d'Ivoire. The children's ward is managed by Paediatricians, medical officers, nurses, health aids and supported by administrative staff. Averagely, about 51,713 patients with varied diseases are reported annually at the out-patient department of the hospital with the total annual admissions for 2013 been 5340 (APH, 2014). According to APH 2014 annual report, the top five causes of outpatient and inpatient mortality are malaria, hypertension, diabetes mellitus, pneumonia and anaemia (APH, 2014). The hospital has both a diagnostic and research Laboratory which undertakes Haematology, Biochemistry, Microbiology analysis and is designated as a training center for health personnel.

Activities of this study were collaborated among three institutions: Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Agogo Presbyterian Hospital and Bernhard-Nocht Institute for Tropical Medicine (BNITM).

KCCR is a joint venture between Kwame Nkrumah University of Science and Technology (KNUST), the Ministry of Health of the Republic of Ghana and BNITM, Hamburg, Germany. The institute has different laboratories: bacteriology, parasitology, immunology, entomology and a molecular laboratory complex. The bacteriology laboratory is equipped with the state of the art equipment such as Class II Biosafety hood,

BACTEC 9050 machines, microscopes, freezers and 37^0C incubator. All the bacteriological activities involving the screening, sensitivity test, DNA extraction and PCR were done at KCCR. Laboratory tests were carried out according to approved Standard Operating Procedures (SOPs) and every activity undertaken in the laboratory was documented.

BNITM is a medical research institution in Hamburg, Germany. It is a renowned centre for tropical medicine. It is resourced to undertake research, training, diagnosis and treatment of tropical and infectious diseases. This institute collaborates with KCCR through KNUST. The genotyping was done in BNITM.

3.4. ETHICAL APPROVAL

The ethical approval for this study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST, Kumasi and Komfo Anokye Teaching Hospital, Ghana. Informed consent of the parents/guardians for the study was done and all parents/guardians of the recruited patients gave informed consent by thumb print.

3.5. STUDY POPULATION

All children age ≤15 years old admitted to the children's ward of the hospital with body temperature ≥38^{o}C were included in this study if they fulfilled the inclusion criteria.

3.6. INCLUSION CRITERIA

- Inpatients aged ≤15 years admitted at APH
- Patients with body temperature ≥38^{o}C
- Children who whose guardians consented to participate in the study.

3.7. EXCLUSION CRITERIA

- Children whose guardians refused to participate in the study

- ❖ All patients above 15 years were excluded from the study

3.8. SAMPLE SIZE

The minimum sample size needed to accurately assess the *S. aureus* nasal carriage was calculated as 180 based on the expected prevalence of nasal colonisation of 14% (Egyir *et al*., 2013). The statistical formula (Daniel, 1999); $SS = Z^2 P (1\text{-}P)/d^2$, where SS = Sample size, Z = standard normal deviation set at 1.96 for 95% confidence interval, *P* = proportion of the target population (*P* =0.14) and d= precision of 5%.

3.9. ENROLMENT INTO THE STUDY

On admission, all children who fulfilled the inclusion criteria were reviewed by our trained paediatric team for sample collection. Children were only recruited when they were admitted to the ward. A purposive sampling technique was used to recruit eligible children. An informed consent form was administered to the prospective participants, explaining in detail the purpose of this nasal carriage research, potential risk or discomfort to the participants and potential benefits. Parents/guardians who agreed voluntarily to take part in the study were made to sign or thumbprint the consent form to provide proof of their willingness to be part of the study. They were assured of the confidentiality of their information. Those who agreed to participate in the study were interviewed by our trained staff and the questionnaire filled-in.

3.10. DEMOGRAPHIC DATA COLLECTION

Demographic data was collected using a questionnaire, which was administered by our trained staff. The socioeconomic variables included: age, sex, village and ethnic group.

3.11. SAMPLE COLLECTION (NASAL SWABS)

The child's head was tilted back gently and steadily from the chin and the sterile Copan transport swab (Copan, Italy) was inserted into the nostrils and rotated against the

turbinate of both anterior nares of each participating child. The nasal swabs were inserted back into the tube containing Stuart-Amine transport media. Each sample was labelled with the unique study identification number (ID), patient name and date of collection and transported to the bacteriology laboratory at KCCR for analysis.

3.12. MICROBIOLOGICAL INVESTIGATIONS

In the laboratory, the nasal swabs were streaked directly on 5% sheep Columbia Blood Agar (BA) (Oxoid CM0331) and Mannitol Salt Agar (MSA) (Oxoid, CM0085)**.** The inoculated plates were then incubated at 37ºC for 48 hours in an aerobic atmosphere.

3.12.1. Culture media.

All media were prepared according to the manufacturers' instruction (Appendix I). The media used in this study included blood agar (Oxoid), Brain Heart Infusion (BHI) broth (BD)Mueller Hinton agar (Oxoid CM0337) and Mannitol salt agar (Oxoid CM0085).

Quality control

The positive control was done with *S. aureus* ATCC 25923 strain while *E. coli* ATCC 25922 was used as a negative control on BA and MSA. The quality of each batch of prepared media was tested by incubating a plate of each media at 37ºC for 24 hours to check for contamination. The accuracy of the antibiotics was also checked once a month.

3.12.2. Identification of *S. aureus*

Bacteria are classified to species level by their shape, Gram reaction and biochemical abilities. *S. aureus* was identified by haemolysis, colony morphology and biochemically by Gram stain, catalase test and confirmed by coagulase test (Cheesbrough, 2002). Isolates were subsequently confirmed by detecting the *nuc*A gene with PCR.

3.12.2.1. Identification of *S. aureus* by colony morphology on mannitol salt agar (MSA)

Staphylococcus aureus from the nasal swabs were presumptively isolated from MSA based on mannitol fermentation. Most other bacteria especially Gram negative bacteria are inhibited by the high concentration of the Sodium Chloride (NaCl). However, some halophilic organisms are able to grow. The fermentation of mannitol by the bacteria produced acid products that changed the pH and the colour of the medium around the colony from pink to yellow. Presumptive *S. aureus* produces yellow colonies whilst other organisms produce reddish purple colonies. Suspected *S. aureus* colonies were selected for further analysis.

3.12.2.2. Identification of *S. aureus* colonies by haemolysis

Haemolysis was observed on blood agar (BA). Since most species cannot be distinguished from one another on the basis of colony morphology within 24 hrs incubation period, colonies were allowed to grow for 48 hours before the primary isolation. Typical *S. aureus* on blood agar has a large, round, creamy smooth golden yellow colonies. Most strains produce beta hemolysis on blood agar plates (Cheesbrough, 2002). These characteristic colonies were sub-cultured for further investigation.

3.12.2.3. Biochemical identification

Presumptive *S aureus* were confirmed using basic biochemical testing procedures which included Gram staining, catalase test and coagulase test. Procedures of these tests are shown in Appendix II.

3.12.2.3.1. Gram stain

The Gram stain differentiates between Gram-negative bacteria and Gram-positive bacteria on the basis of the differential interactions of the cell wall components of these

two groups of bacteria with Gram's reagents. The thick peptidoglycan cell wall of Gram-positive bacteria retains the crystal violet colour after decolouration with acetone leading to a blue appearance under the light microscope. Gram-negative bacteria do not hold the crystal violet-iodine complex but absorbs the carbolfucshion or the safranin and therefore appear red or pink in colour.

3.12.2.3.2. Catalase test

The enzyme catalase is a virulence factor produced by Staphylococci, which degrades the microbicidal hydrogen peroxide (H_2O_2) into H_2O and O_2. Catalase test is important in the differentiation of Staphylococci from Streptococci.

PRINCIPLE: Organisms capable of producing catalase are able to reduce hydrogen peroxide with the production of oxygen. When catalase-positive organisms are exposed to hydrogen peroxide (H_2O_2), oxygen is released and this is being detected by the formation of bubbles.

3.12.2.3.3. Coagulase test (Bannerman *et al*., 2003)

Staphylococcus aureus is differentiated from other staphylococci by producing the enzyme coagulase that clots plasma. *Staphylococcus aureus* produces two main types of coagulase enzymes; bound coagulase and free coagulase. The bound coagulase enzyme is tested by the slide coagulase test. The tube coagulase which detects the free coagulase was used in this study.

PRINCIPLE: *Staphylococcus aureus* produces a diffusible substance designated free coagulase, which can clot plasma. The test organism is incubated in medium containing rabbit plasma. If free coagulase is produced, it reacts with a component of plasma called coagulase-reacting factor, leading to a fibrin clot.

3.13. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antibiotic susceptibility test was carried out using the Kirby- Bauer's disk diffusion method. This method was performed according to the standards of Clinical and Laboratory Standard Institute (CLSI, 2014). Bacterial suspension in a physiological saline adjusted to 0.5 McFarland was used for the inoculation. A sterile cotton swab was used to collect and spread the inoculum on the entire surface of a MH agar. First swabbing the entire surface and then turning the plate at a perpendicular angle and repeating the swabbing to achieve a lawn of bacterial growth. The filter papers disk impregnated with a specific amount of antimicrobial concentration were placed in the inoculated surface of Mueller-Hinton agar (MH) medium. The disks were placed on the MH using the disk dispenser (Oxoid), which dispenses 6 discs with equal distance apart from each other on a 90 mm diameter plate. The plates were incubated in an aerobic condition at 35-37°C for 24 h in an inverted position within 15 minutes of the placement of the disks. There was no growth in areas where the concentration of the drug inhibited the growth of the bacteria. The diameter of the zones of complete inhibition was measured in millimetres (mm).

The antimicrobial agents that were tested included the following: cefoxitin (30µg), clindamycin (2µg), erythromycin (15µg), teicoplanin (10µg), penicillin (10µg), linezolid (30ug), ciprofloxacin (5µg), tetracycline (30µg), trimethoprimsulfamethoxazole (25µg) and gentamicin (10µg) using commercial discs from Oxoid. The susceptibility test results were reported categorically as Resistant (R), Intermediate (I) or Sensitive (S). The cefoxitin (30µg) disc was used to presumptively screen for the presence of MRSA. Multidrug resistance (MDR) was defined as resistance to at least three distinct antimicrobial classes (Magiorakos *et al.*, 2012).

CLSI (2014) Breakpoints

Penicillin: ≤28mm (R), ≥29mm (S)

Clindamycin: ≤14mm (R), 15mm-20mm (I), ≥21mm (S)

Gentamicin: ≤12mm (R), 13mm-14mm (I), ≥15mm (S)

Cefoxitin: ≤ 21mm (R), ≥22mm (S)

Tetracycline: ≤14mm (R), 15mm-18mm (I), ≥19mm (S)

Trimethoprim/Sulfamethoxazole: ≤10mm (R), 11mm-15mm (I), ≥16mm (S)

Teicoplanin: ≤10mm (R), 11mm-13mm (I), ≥14mm (S)

Linezolid: ≥21mm (S)

3.14. MOLECULAR TECHNIQUES

3.14.1. DNA extraction

Genomic DNA from the isolates was extracted from an overnight culture of pure colonies grown in BHI broth using the Presto Spin D Bug kit according to the instructions of the manufacturer.

The overnight culture grown in BHI broth was centrifuged in a microcentrifuge (Eppendorf centrifuge 5417R) at 13,000 rpm for 2 min. The supernatant was removed and 50µl buffer RS incl. 5µl lysostaphin was then added to the cell pellet. It was mixed and incubated at 37°C for 30 min. Resuspension of cells was accelerated by vortexing in 5s intervals at full speed and repeatedly pulling the tube along the holes of a sample rack with some pressure. An amount of 250µl of buffer CH was added and immediately vortexed for 5 seconds. The suspension was stirred with a pipette tip for homogenization and allowed to stand until visible lysis has occurred (at least 5 min) with the suspension becoming clear and/or viscous. An amount of 200µl of buffer AB was added, vortexed

for 5 seconds and the solution was transferred to a spin column. The spin column was centrifuged at 13,000 rpm for 30 s. The flow-through was then discarded. To degrade RNA, 200µl of buffer RB including RNase A was transferred to the spin column and incubated for 10min. A volume of 400µl buffer WB was added to the spin column and centrifuge at 13,000 rpm for 30 s. The flow-through was discarded and the spin column was washed with 400µl of 70 % ethanol by centrifugation at 13,000 rpm for 3 min and the flow-through was discarded. The spin column was transferred to a 1.5ml tube and a preheated 75µl buffer EB at 70 °C was placed in the center of the column. The lid was closed and incubated for 1 min. Thereafter, it was centrifuged at 13,000 rpm for 1 min to elute the DNA. The extracted DNA was then stored at -20 °C.

3.14.2. Quality control of the DNA extracted

The quality of the DNA was done by agarose gel electrophoresis (gel preparation in 3.16). Amount of 5ul of the extracted DNA preparation was added to 2ul of gel loading buffer (6x bromophenol blue). It was mixed and transferred to a 1.5% gel slot and run in 0.5 x TBE (tris boric electrophoresis buffer at 100V for 40 min. The resulting DNA bands obtained were visualized and photograph taken under UV illumination (Bio Rad, Hercules, CA).

3.14.3. Polymerase Chain Reaction (PCR)

The PCR was performed in a total reaction volume of 50µl made up of the genomic DNA, PCR H_2O, primers, HotstarTaq polymerase (QIAGEN), 2mM MgCl2 and dNTPs (dATP, dCTP, dGTP, dTTP). The protocol for amplification was set in the PCR thermocycler (QTQ-Cycler 96, Hain lifescience Germany) depending on the primers used. After the PCR process, the amplified products were separated by 1.5% agarose gel. The positive control was ATCC 25923 and the negative control was RNase free water.

3.14.4. Preparation of the gel

Gel electrophoresis separates DNA on the basis of their sizes and charge. When an electric field is applied, the DNA moves through the Agarose matrix. The 1.5% Agarose gel was prepared by weighing 1.2g of the Agarose and dissolving it into 80ml of 0.5x TBE (Tris-Borate-EDTA) and heated in a microwave for 1.5 min to dissolve it. During this time, the tray was prepared by placing cello tape on both ends of the tray and a comb placed into the tray. It was allowed to cool to a temperature of about 40^0C before adding 4ul of ethidium bromide to it. This mixture was mixed well and poured into the prepared casting tray. It was allowed to set for at least 20mins. Once the gel was set, the comb was carefully removed.

3.14.5. Gel electrophoresis

The casting tray was placed in the electrophoresis chamber containing 0.5x TBE running buffer and 10µl of the appropriate amplicon was mixed well with 2ul of loading dye and loaded into each of the well. A volume of 4ul generuler 100bp ladder (Peqlab, VWR) was placed into the first well to serve as a standard for estimating the size of the DNA fragment. The positive control (ATCC 25923) and negative control (water) were placed in the last two wells. The electrophoresis chamber was connected to the power supply and electrophoresis was run at 100Vfor 40min. The DNA bands were viewed by illumination and images photographed under the UV light.

3.14.6. Polymerase Chain Reaction (PCR) primers

All the oligonucleotide primers used in detecting various genes were obtained from Eurofins, Germany and they are listed in the table below.

Table 3.1: The primers used in this study

Gene	Primer Sequence	Amplicon Size	Source of primers
*nuc*A- 1 *nuc*A- 2	5' – GCGATTGATGGTGATACGGTT – 3' 5'–AGCCAAGCCTTGACGAACTAAAGC – 3'	279 bp	Aiken *et al*., 2014
spa-1113f *spa*-1514r	5' – TAAAGACGATCCTTCGGTGAGC –3' 5, – CAGCAGTAGTGCCGTTTGCTT – 3'	Variable between 300 - 500 bp	Shakeri and Ghaemi, (2014)
*Mec*A-P4 *MecA*-P7	5'–TCCAGATTACAACTTCACCAGG– 3' 5'–CCACTTCATATCTTGTAACG– 3'	162 bp	Stegger *et al*,. (2012)
*Mec*C-F *Mec*C-R	5'–GAAAAAAAGGCTTAGAACGCCTC– 3' 5'–GAAGATCTTTTCCGTTTTCAGC– 3'	138 bp.	Stegger *et al*,. (2012)
PVL-for PVL-rev	5'– ATCATTAGGTAAAATGTCTGGAA– 3' 5'– GCATCAASTGTATTGGATAGCAAC– 3'	433 bp	Lina *et al*., (1999)

3.14.7. Molecular confirmation of *S. aureus* by detection of *nuc*A gene.

S. aureus strains produce an extracellular thermostable nuclease which is coded for by the *nuc*A gene (Brakstad *et al*., 1992). Amplification of the gene was done for *S. aureus* confirmation. Strain ATCC 25923 was used as the positive control and RNase free water was used as negative control. The PCR protocol was carried out in a 50µl total reaction volume containing a mixture of 2µl of DNA template, 5µl of 10×PCR buffer, 35.75µl of PCR H_2O, 1µl of a 1.25mM mixture of deoxynucleosidetriphophate, 4µl of 25mM MgCl2, 0.25µl of 5U of HotstarTaq DNA polymerase and 1ul each of 100pmol of *nuc*A primer pairs (Eurofins, Germany).

The cycling conditions included an initial denaturation at 94°C for 15min, followed by 30 cycles of (denaturation at 94°C for 45s, annealing temperature at 55°C for 45s, and

extension at 72°C for 45s) and a final extension at 72°C for 10 min. It was left on hold at 4°C. The PCR product (10µl) was mixed with 2µl of the loading buffer (Bromophenol blue) and added to the wells created on the 1.5% (wt/vol) Agarose gel. The gel electrophoresis was run at 100mV for 40min. The DNA bands were photographed under UV illumination (Plate 3.1).

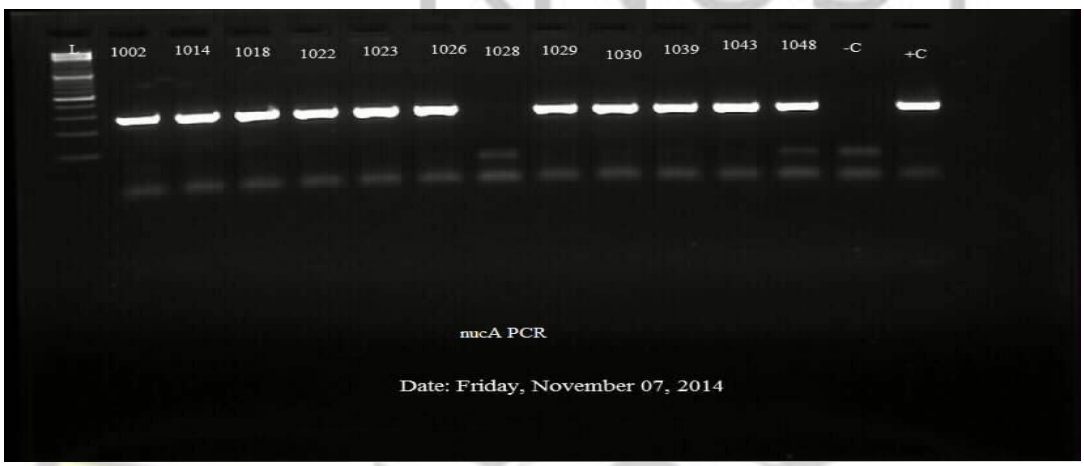

Plate 3.1: Amplification of the *nuc*A gene

L: 100bp molecular weight marker; 1002, 1014, 1018, 1022, 1023, 1026, 1029, 1030, 1039, 1043, 1048 with the prefix 70 are the positive samples. 1028 was coagulase and *nuc*A negative (Just for test), –C: Negative control/ water and +C: ATCC25923 (MSSA) (positive control).

3.14.8. Amplification of the *mec*A and *mec*C genes

Resistance to methicillin was confirmed by *mecA* and *mec*C PCR. The PCR protocol for each of the primer set of *mecA* and *mec*A was performed in separate 50µl total volume of PCR reaction mixture containing 2µl of DNA template, 5µl of 10×PCR buffer, 35.75µl of PCR H_2O, 1µl of a 1.25 mM mixture of deoxynucleosidetriphophate (dNTPs), 4µl of 25mM MgCl2, and 0.25µl of 5U of HotstarTaq polymerase (QIAGEN, Hain lifescience Germany) and 1µl (100pmol) each of the primer set. The primer pair sequences are shown in Table 3.1.

The cycling conditions included an initial denaturation at 94°C for 15min, followed by 30 cycles of (denaturation at 94°C for 45s, annealing at 55°C for 45s and extension at 72°C for 45s) and a final extension at 72°C for 10 min. It was left on hold at 4°C. A volume of 10µl of the amplified product was mixed with 2µl of the loading dye and loaded to the 1.5% (wt/vol) Agarose gel. The gel electrophoresis was run at 100V for 40min. The DNA bands were photographed under UV illumination (Plate 3.2).

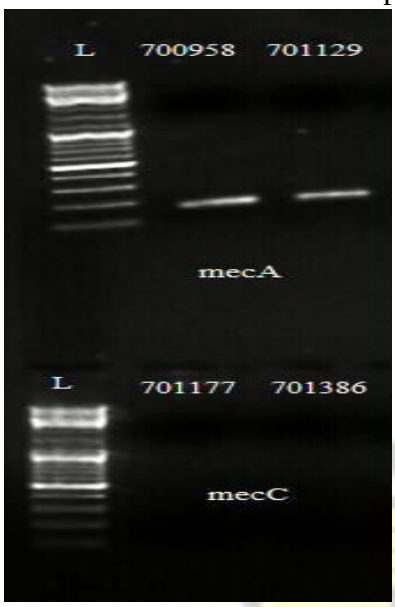

Plate 3.2: Amplification of the *mec*A and *mec*C genes

L: 100bp molecular weight marker; 700958 and 701129 were *mec*A positive and there was no *mec*C positive.

3.15. DETECTION OF PANTON-VALENTINE LEUKOCIDIN (PVL) TOXIN

The *lukS-PV* and *lukF-PV* genes which encode Panton-Valentine leukocidin, was detected by PCR as described (Lina *et al*., 1999). RNase free water was used as a negative controls and the positive control was sta 635/636 (PVL-positive). The PCR was performed in a total reaction volume of 50µl containing a mixture of 2µl of DNA template, 5µl of 10×PCR buffer, 35.75µl of PCR H_2O, 1µl of a 1.25mM mixture of deoxynucleosidetriphophate (dNTPs), 4µl of 25mM MgCl2, and 0.25µl of 5U of

HotstarTaq polymerase (QIAGEN, Hain lifescience Germany) and 1µl (100pmol) each of the forward and reversed primers.

The cycling conditions included an initial denaturation at 94°C for 15min, followed by 30 cycles of (denaturation at 94°C for 45s, primer annealing at 55°C for 45s, extension at 72°C for 45s) and a final extension at 72°C for 10 min. It was left on hold at 4°C.

The PCR product (10µl) was mixed with 2µl of the loading buffer and loaded to the wells created on the 1.5% (wt/vol) Agarose gel already stained with ethidium bromide. The gel electrophoresis was run at 100V for 40min. The resulting DNA bands were viewed and photographed under UV light (Plate 3.3).

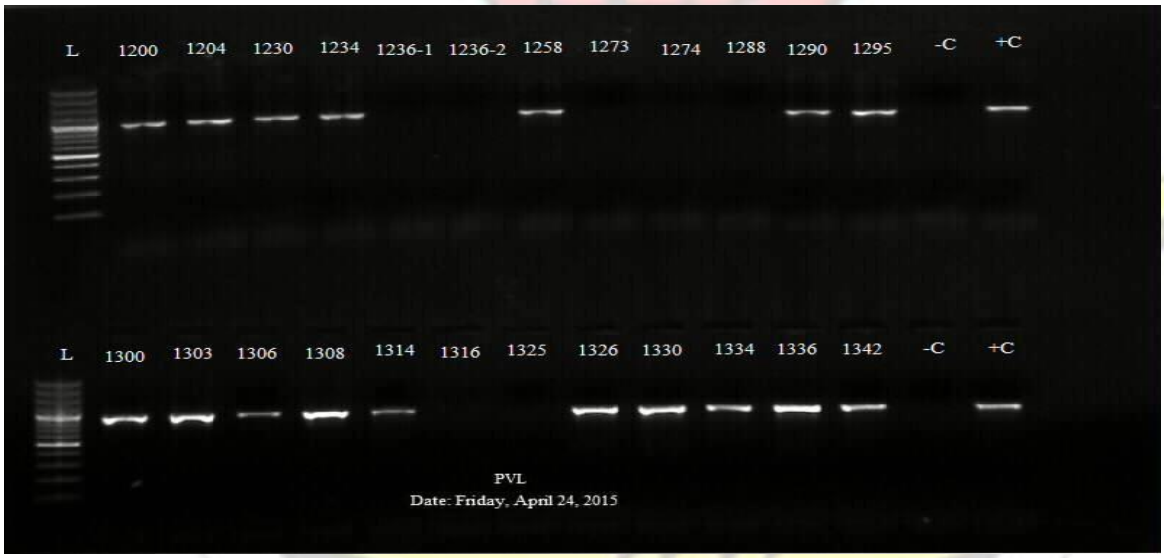

Plate 3.3: Amplification of the PVL toxin encoding genes

L: 100bp molecular weight marker; 1200, 1204, 1230, 1258, 1290, 1295, 1300, 1304, 1308, 1314, 1326, 1330, 1334, 1336, 1342 with the prefix 70 were the positive for PVL. -C: Negative control/ water and +C: (positive control)

3.16. MOLECULAR TYPING OF *S. AUREUS* STRAINS

3.16.1. Staphylococcal protein A (spa) typing

The variable X region of the staphylococcal protein A (*spa)* gene which is made up of 24-bp repeats was amplified by PCR (Shakeri and Ghaemi, 2014). The total reaction volume of the PCR was 50µl containing 2µl of DNA template, 5µl of 10×PCR buffer, 35.75µl of PCR H2O, 1µl of a 1.25mM mixture of deoxynucleosidetriphophate, 4µl of 25mM MgCl2, and 0.25µl of 5U of HotstarTaq DNA polymerase (Qiagen) and 1µl (10pmol) each of the spa primers. The *spa* region was amplified using the primers in table 3.1. The following PCR cycling conditions were used to amplify the target DNA: an initial denaturation at 94°C for 15min, followed by 30 cycles of (denaturation at 94°C for 45s, primer annealing at 55°C for 45s, extension at 72°C for 45s) and a final extension at 72°C for 10 min. It was left on hold at 4°C. The amplified product (10µl) was added to 2µl loading dye (Bromophenol blue) and added to the wells created on the 1.5% (wt/vol) Agarose gel stained with ethidium bromide. The gel electrophoresis was run at 100mV for 40min. The DNA bands were then viewed and photographed under UV illumination. The *S. aureus* strain ATCC 25923 was used as the positive control (plate 3.4).

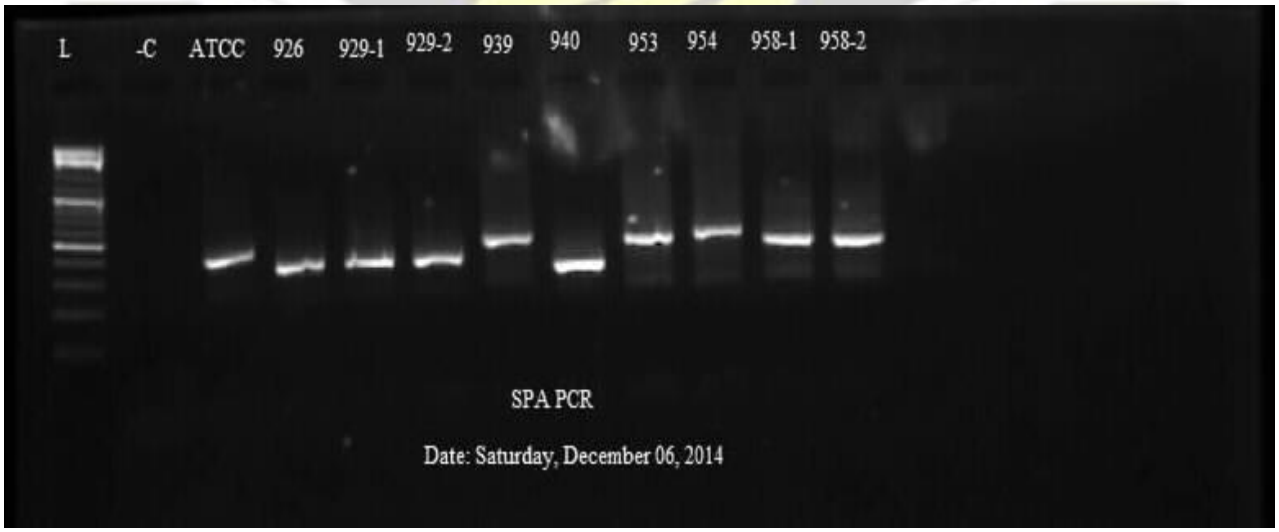

Plate 3.4: Amplification of the *spa* gene

3.16.2. Purification of *spa* PCR product

The amplified *spa* product was purified using the GeneJET™ PCR Purification Kit before it was sent to Seqlab for sequencing. The purification was done according to the protocol from the manufacturer.

Procedure: An equal volume of the Binding Buffer was added to the PCR product (40µl of Binding Buffer was added to 40µl of the PCR product). The colour of the mixture was yellow. The mixture was then transferred to the purification column, centrifuged for 60s and the flow-through was discarded. An amount of 700µl wash buffer diluted with the ethanol was added to the purification spin column. It was centrifuge for 60s and the flow-through was discarded. The purification column was placed back into the collection tube. The empty GeneJET purification spin column was centrifuged for another 1 min to completely remove any residual wash buffer since the presence of ethanol in the DNA sample may inhibit subsequent reactions. The purification column was then transferred to a new 1.5ml Eppendorf tube and 50µl of Elution Buffer was added to the center of the purification spin column membrane. It was centrifuged for 1 min to elute the purified DNA and the DNA was store at -20°C.

3.16.3. DNA Sequencing and *spa* typing

The DNA sequences were obtained by seqlap (Applied Biosystems, Foster City, Calif) which uses Sanger sequencing method. An amount of 12µl of the purified *spa* amplicon was sent in pre labelled sequencing tubes to seqlap for sequencing. The *spa* sequence typing was done with the Ridom StaphType standard protocol

(http://www.3.ridom.de.doc/Ridom_spasequencing.pdf) and the Ridom *spa* server which automatically analyses the *spa* repeats and assigns *spa* types (http://spa.ridom.de/index.shtml). The *spa* types were obtained using the Ridom StaphType software version 1.5 betas (Ridom GmbH, Wurzburg, Germany) at Uniklinikum, Bonn-Germany.

3.17. EPIDEMIOLOGICAL ANALYSIS

The data was entered into Microsoft Excel 2013 and analysed with STATA 12 (©4D College Station, Texas, United Sates). Microsoft Excel 2013 was used for the graphs. Frequencies were obtained and percentages were calculated for study variables. Descriptive statistics were used to determine the outcome of the variables.

CHAPTER FOUR

RESULTS

4.1. DEMOGRAPHIC CHARACTERISTICS OF THE PARTICIPANTS

A total of 545 nasal swabs were obtained from the children. Majority of the recruited patients were males (53.0%). Patients' ages were from 0-14 years. The mean age of the study participants was 3.2 years. The age distribution indicates that the 1-5 years age group had the highest number of participants (65.9%) and the age group 11-15 years had the least number of recruitments of 3.7%. Study participants came from 38 different villages. Villages with at least ten recruited participants were included in the analysis. Majority of the participants; 205 (37.6%) were from Agogo. Akans represented 60.6% of the participants whiles the least (0.4%) were Ga (Table 4.1).

4.2. PREVALENCE OF *S. AUREUS* AMONG PATIENTS

A total of 120 *S. aureus* were isolated from the nasal swabs obtained from the 545 patients. All the *S. aureus* isolates were confirmed by detecting the *nuc*A gene via

polymerase chain reaction. Thus, the prevalence of *S. aureus* among the hospitalized children in the Agogo Presbyterian hospital was estimated to be 22.0% (Figure 4.1). Among these 120 isolates, 97.3% were MSSA and 1.7% were MRSA as presented in Figure 4.2.

Table 4.1. Demographic characteristics of the recruited patients

Characteristics of children	Category	n (%) Patients (N=545)
Sex	Male	289(53.0)
	Female	256(47.0)
Village	Agogo	205(37.6)
	Konongo	30(5.5)
	Ananekrom	28(5.1)
	Hwidiem	23(4.2)
	Bahankra	18(3.3)
	Bebuso	13(2.4)
	Domeabra	11(2.0)
	Pekyerekye	10(1.8)
	Others	207(38.0)
Age Group of Children	<1 year	83(15.2)
	1-5 years	359(65.9)
	6-10 years	83(15.2)
	11-15 years	20(3.7)
Ethnicity	Akan	330(60.6)
	Northerner	202(37.1)
	Ewe	9(1.7)
	Ga	2(0.4)

	Missing data	2(0.4)

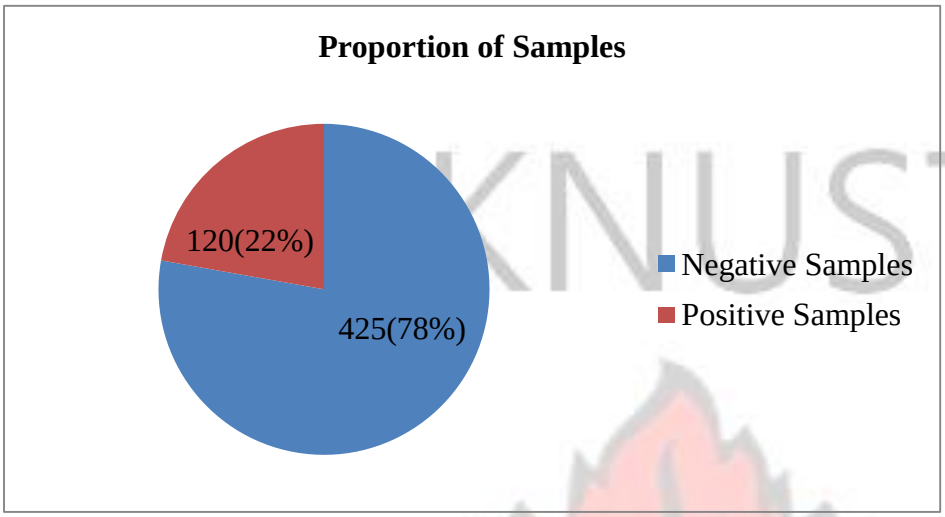

Figure 3.1: Proportions of positive and negative samples

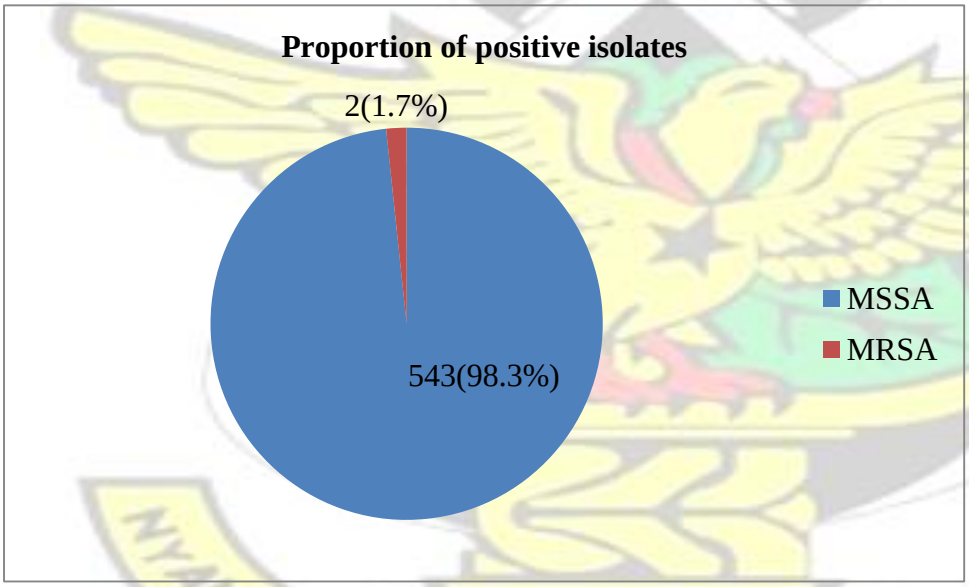

Figure 4.2: Proportion of MSSA and MRSA among positive samples

4.3. DEMOGRAPHIC CHARACTERISTICS AND NASAL CARRIAGE OF *S. AUREUS*

More females (23.8%) were colonised with *S. aureus* as compared with (20.8%) of the males. Participants from the age group 6-10 years recorded the highest nasal carriage

(36.1%) of *S. aureus* while the age group 10-14 years had the least nasal carriage (15.0%). 22.0% of those from Agogo were nasal carriers. Meanwhile those from Bebuso and Pekyerekye had the highest nasal carriage of 30.8% and 30.0% respectively. Majority of the Ewe (33.2%) were nasal carriers followed by the Northerners (23.8%).

Table 4.2: *S. aureus* carriers by demographic characteristics.

Characteristic	Category	Number positive in the category (%)	Overall % positive (N=120)
Sex	Male	59/289(20.4)	49.2
	Female	61/256(23.8)	50.8
Village	Agogo	45/205(22.0)	37.5
	Konongo	6/30(20.0)	5.0
	Ananekrom	5/28(17.9)	4.2
	Hwidiem	6/23(26.1)	5.0
	Bahankra	5/18(27.9)	4.2
	Bebuso	4/13(30.8)	3.3
	Domeabra	2/11(18.2)	1.7
	Pekyerekye	3/10(30.0)	2.5
	Others	46/207(22.2)	38.3
Age group/years	<1 year	13/83(15.0)	10.8
	1-5years	74/359(20.0).	61.7
	6-10 years	30/83(36.0)	25.0
	11-15 years	3/20(15.0)	2.5
Ethnicity	Akan	69/330(20.0)	57.5
	Northern	48/202(23.0)	40.0
	Ewe	3/9(33.0)	2.5

Ga	0/2(0.0)	0.0
Missing	0/2(0.0)	0.0

4.4. PREVALENCE OF METHICILLIN-RESISTANCE *S. AUREUS*

Out of the 120 *S. aureus* isolates, 2(1.7%) was cefoxitin resistant and they were subsequently confirmed as MRSA by detecting the *mec*A gene by PCR. Even though *mec*C was tested, there was no *mec*C found. Thus, the prevalence of MRSA among the *S. aureus* was 1.7%. The MRSA isolates were sensitive to clindamycin, gentamicin, linzolid and teicoplanin. The characteristics of the MRSA are given in Table 4.3. The two MRSA isolates were both resistant to cefoxitin, penicillin and tetracycline. One was from a 3 year old male, resident of Agogo which harboured a PVL positive strain belonging to the *spa* type t1096. The other MRSA was isolated from a 7 year old male, resident in Bahankra with PVL negative strain that belongs to *spa* type t4454. Both of them were northerners.

Table 4.3: Characteristics of the MRSA isolates

ID	Age	Sex	Village	Ethnicity	*spa* type	PVL	Antibiotic resistance
700958	3	Male	Agogo	Northerner	t1096	Positive	FOX30, P10, TE30
701129	7	Male	Bahankra	Northerner	t4454	Negative	FOX30, P10, TE30

FOX30: cefoxtin, TE30: tetracycline, P10: penicillin

4.5. ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF THE *S. AUREUS* ISOLATES

The antimicrobial susceptibility pattern of the 120 *S. aureus* isolates from the nasal samples is shown in Table 4.4. Among the 120 *S. aureus*, penicillin recorded the highest resistance of 96.7% followed by tetracycline with 52.5% and erythromycin with 6.7%. Of all the *S. aureus* isolates, 1.7% (n=2) were cefoxitin resistant. Few of the isolates exhibited intermediate resistance to ciprofloxacin (2.5%), erythromycin (6.7%), gentamycin (0.8%) and clindamycin (4.2%). However, all the isolates were 100% susceptible to oxazolidinones (linezolid) and glycopeptides (teicoplanin) (Table 4.4).

Nine antibiotypes (combination of resistance to antibiotics) were established from this study. The most prevalent was penicillin and tetracycline (P10R TE30R) accounting for 42.5% (n=51) of the total isolates; followed by penicillin, tetracycline and erythromycin (P10R TE30R E15R) accounting for 5% (n=6) of total isolates. However, four isolates (3.3%) were susceptible to all the antimicrobial drugs tested and 11(9.2%) exhibited multiple drug resistance (Table 4.5).

Table 4.4: Antimicrobial susceptibility profile of the 120 *S. aureus* isolates

	Carrier isolates (N=120)		
Antibiotic	**%Resistance**	**%Intermediate**	**%Sensitive**
Penicillin	96.7(116)	0(0)	3.3(4)
Tetracycline	52.5(63)	0(0)	47.5(57)
Erythromycin	6.7(8)	6.7(8)	86.7104)
Trimethoprim/Sulfamethoxazole	2.5(3)	0(0)	97.5(117)
Clindamycin	1.7(2)	4.2(5)	94.2(113)
Cefoxitin	1.7(2)	0(0)	98.3(118)
Ciprofloxacin	0(0)	2.5(3)	97.5(117)
Gentamycin	0(0)	0.8(1)	99.2(119)

Teicoplanin	0(0)	0(0)	100(120)
Linezolid	0(0)	0(0)	100(120)

Table 4.5: Resistance Phenotypes of *S. aureus* isolates from carriage

Resistance phenotypes	Number of isolates
None	4
P10	50
TE30	1
P10, SXT25	2
P10, TE30	51
P10, E15	1
P10, TE30, E15	6
P10, TE30, FOX30	2
P10, TE30, SXT25	1
P10, TE30, DA2	1
P10, TE30, E15, DA2	1

P10: penicillin; SXT25: trimethoprim/sulfamethoxazole; E15: erythromycin; FOX30: cefoxitin; DA2: clindamycin; TE30: tetracycline

4.6. IDENTIFICATION OF PVL AMONG *S. AUREUS* ISOLATES

Out of the 120 *S. aureus* isolates screened for the presence of PVL, 69 (57.5%) were PVL positive (Figure 4.3).

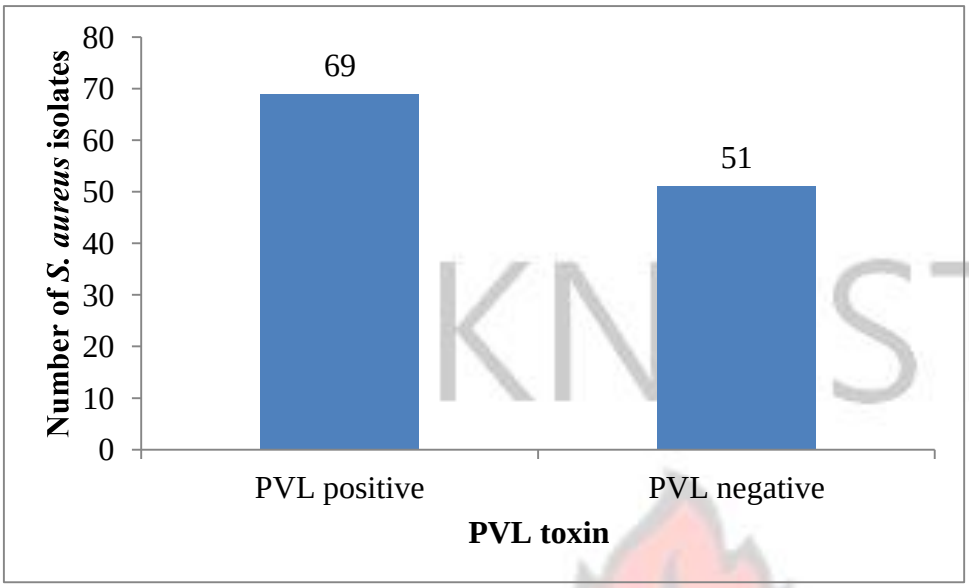

Figure 4.3: Prevalence of PVL toxin producing *S. aureus*

4.7. GENOTYPE DIVERSITY OF THE ISOLATES ESTABLISHED BY *SPA* TYPING

Thirty-five different *spa* types were identified in this study. The most prevalent *spa* types were t355 (20%), t084 (14.9%) and t939 (10.7%). Two novel *spa* types: t15727 and t15728 were discovered and annotated during the study (Table 4.6).

Table 4.6: The distribution of *spa* types among the *S. aureus* isolates

***spa* type (N=35)**	**n(%) *S. aureus* isolates (N=120)**
t355	24(20.0)
t084	18(14.9)
t939	13(10.7)
t311	7(5.8)
t314	7(5.8)
t4454	4(3.3)
t1299	4(3.3)
t008	3(2.5)
t1123	3(2.5)
t127	3(2.5)
t363	3(2.5)

t1096	3(2.5)
t1114	2(1.7)
t4104	2(1.7)
t6063	2(1.7)
t616	2(1.7)
t537	2(1.7)
t021	1(0.8)
t085	1(0.8)
t1172	1(0.8)
t1476	1(0.8)
t1510	1(0.8)
spa type (N=35)	**n(%) _S. aureus_ isolates (N=120)**
t157	1(0.8)
t15727*	1(0.8)
t15728*	1(0.8)
t186	1(0.8)
t2147	1(0.8)
t318	1(0.8)
t346	1(0.8)
t385	1(0.8)
t450	1(0.8)
t451	1(0.8)
t5047	1(0.8)
t591	1(0.8)
t645	1(0.8)

4.8. THE DISTRIBUTION OF *SPA* TYPES AMONG VILLAGES

The study participants were residents of 38 different villages. For simple and clear presentation of the results, villages with at least ten participants were selected. The rest of the villages were grouped as others. Agogo recorded the presence of most of the *spa* types identified in this study. The *spa* types t355, t084 and t939 were widely distributed

in these selected catchment areas with *spa* types t021, t1172, t15727*, t15728*, t186, t2147 and t645 only isolated from residents of Agogo. Table 4.7 below shows distribution of *spa* types among the villages of residence of study participants.

Table 4.7: Distribution of *spa* types among villages with at least ten participants

spa type	AGO (45)	KON (6)	ANA (3)	HWI (6)	BAH (5)	BEB (4)	DOE (2)	PEK (3)	Others (22)
t355	7	1	2	-	2	1	-	-	11 t084	8
	1	-	1	1	2	-	-	5 t939	5	1	-
	2	-	-	1	-	4 t311	2	-	-	1	1
	-	-	1	2 t314	2	-	-	-	-	-	-
	5 t4454		1	-	-	-	1	-	-	-	2
t1299		2	-	1	-	-	-	-	-	1 t008	2
	-	-	1	-	-	-	-	- t1123		2	-
	-	-	-	-	-	-	1 t127	3	-	-	-
	-	-	-	-	- t363	2	-	-	-	-	-
	-	-	1 t1096		1	-	-	-	-	-	-
	-	2 t1114		1	-	-	-	1	-	-	-
t4104		-	-	-	-	-	-	-	-	2 t6063	
	-	1	-	-	-	-	1	-	- t616	-	-
	-	-	-	-	-	2	- t537	1	1	-	-
	-	-	-	-	- t021	1	-	-	-	-	-
	-	-	- t085	-	-	-	-	-	-	-	-
	1 t1172		1	-	-	-	-	-	-	-	-
t1476		-	-	-	-	-	-	-	-	1 t1510	
	-	-	-	-	-	-	-	-	1 t157	-	-
	-	-	-	-	-	-	1 t15727		1	-	-
	-	-	-	-	-	- t15728		1	-	-	-
	-	-	-	-	- t186	1	-	-	-	-	-
	-	-	- t2147		1	-	-	-	-	-	-

-	-	t318	-	-	-	1	-	-	-	-	-
t346	-	-	-	-	-	-	-	-	1		

spa type	AGO (45)	KON (6)	ANA (3)	HWI (6)	BAH (5)	BEB (4)	DOE (2)	PEK (3)	Others (22)
t385	-	-	-	-	-	-	-	-	1
t450	-	-	-	-	-	-	-	-	1
t451	-	-	-	-	-	-	-	-	1
t5047	-	-	-	-	-	-	-	-	1
t591	-	1	-	-	-	-	-	-	-
t645	1	-	-	-	-	-	-	-	-

AGO: Agogo, KON: Konongo, HWI:Hwidiem, BAH: Bahankra, BEB: Bebuso, DOE: Domeabra, PEK: Pekyerekye

4.9. ANTIMICROBIAL RESISTANCE AMONG *SPA* TYPES

Table 4.8 shows antimicrobial resistance among the various *spa* types identified in this study. None of the *spa* types were resistant to linezolid, teicoplanin, ciprofloxacin. Each of the *spa* types had at least 50% resistance to penicillin. Ten *spa* types (t021, t084, t1096, t1123, t311, t314, t355, t4454, t450, t451) exhibited MDR as shown in Table 4.9 and Table 4.10. In all the *spa* types, the highest proportion and frequently observed resistance was recorded for penicillin followed by tetracycline.

Table 4.8: Percentage of resistance among *S. aureus spa* types

	Antibiotic resistance (%)					
spa type	FOX30	SXT25	E15	TE30	DA2	P10
t008	-	-	-	-	33.3	66.7
t021	-	-	100	100	-	100
t084	-	5.6	5.6	66.7	-	100
t085	-	-	-	-	-	100
t1096	33.3	-	-	33.3	-	33.3
t1114	-	-	-	50	-	100

t1123	-	-	33.3	100	33.3	66.7
t1172	-	-	-	100	-	100
	Antibiotic resistance (%)					
spa type	FOX30	SXT25	E15	TE30	DA2	P10
t127	-	-	-	33.3	-	100
t1299	-	-	-	25	-	75
t1476	-	-	-	-	-	100
t1510	-	-	-		-	100
t157	-	-	-	100	-	100
t15727	-	-	-	-	-	100
t15728	-	-	-	-	-	100
t186	-	-	-	100	-	100
t2147	-	-	-	100	-	100
t311	-	-	14.3	71.4	14.3	100
t314	-	14.3	14.3	57.1	-	100
t318	-	-	-	-	-	100
t346	-	-	-	100	-	100
t355	-	-	8.3	70.8	-	100
t363	-	-	-	-	-	100
t385	-	-	-	-	-	100
t4104	-	-	-	100	-	100
t4454	100	-	-	100	25	100
t450	-	-	100	100	-	100
t451	-	100	-	100	100	100
t5047	-	-	-	100	-	100
t537	-	-	-	-	-	100
t591	-	-	-	-	-	100
t6063	-	-	-	50	-	100
t616	-	-	-	-	50	1(50)
t645	-	-	-	100	-	100
t939	-	-	-	7.7	7.7	100

P10: penicillin; SXT25: trimethoprim/sulfamethoxazole; E15: erythromycin; FOX30:

cefoxitin; DA2: clindamycin; TE30: tetracycline

Table 4.9. Characterization of the *spa* types resistance phenotypes

spa type	Antibiotic resistance phenotype
t008	P10 /P10,DA2
t021	E15,TE30,P10
t084	P10/P10,TE30/P10,TE30,E15/P10,SXT25
t085	P10
t1096	FOX30,TE30,P10
t1114	DA2,P10
t1123	P10,TE30/P10,TE30,E15
t1172	TE30,P10
t127	TE30,P10
t1299	TE30,P10
t1476	P10
t1510	P10
t157	TE30,P10
t15727	P10
t15728	P10
t186	TE30,P10
t2147	TE30,P10
t311	P10,TE30/P10,E15
t314	P10,TE30/P10,TE30/P10,SXT25
t318	P10
t346	TE30,P10
spa type	Antibiotic resistance phenotype

t355	P10,TE30/P10,TE30,E15
t363	P10
t385	P10
t4104	TE30,P10
t4454	FOX30,P10,TE30/P10,TE30/P10,TE30
t450	E15,TE30,P10
t451	SXT25,TE30,P10
t5047	TE30,P10
t537	P10
t591	P10
t6063	P10,TE30
t616	DA2,P10
t645	TE30,P10
t939	TE30,P10

Table 4.10: The resistance phenotypes of MDR *spa* types

spa type	Resistance phenotypes of MDR
t021	E15,TE30,P10
t084	SXT25,E15,TE30,P10
t1096	FOX30,TE30,P10
t1123	E15,TE30,DA2,P10

spa type	Resistance phenotypes of MDR
t311	E15,TE30,DA2,P10
t314	SXT25,E15,TE30,P10
t355	E15,TE30,P10
t4454	FOX30,TE30,DA2,P10
t450	E15,TE30,P10
t451	SXT25,TE30,P10

4.10. DISTRIBUTION OF PVL TOXIN AMONG *SPA* TYPES

Spa type t355 recorded 20(29%) of the PVL positive isolates followed by t084 which had 12(17.4%). Fifteen *spa* types: t008, t021, t085, t1476, t1510, t157, t15727*, t15728*, t2147, t450, t537, t591, t6063, t616, t645 were PVL negative. The *spa* type t314 were all PVL positive.

Table 4.11 Distribution of PVL toxin among *spa* types

spa type	n(%) PVL *S. aureus* isolates (N=69)
t008, t021 ,t085, t1476, t1510, t157, t15727, t15728, t2147, t450, t537, t591, t6063, t616, t645	0(0)
t084	12(17.4)
t1096	3(4.4)
t1114	1(1.5)
t1123	2(2.9)

t1172	1(1.5)
t1299	3(4.5)
t186	1(1.5)
t311	5(7.3)
t314	7(10.1)
t318	1(1.5)
t346	1(1.5)
t355	20(29)
t363	3(4.4)
t385	1(1.5)
t4104	1(1.5)
t4454	1(1.5)
t451	1(1.5)
t5047	1(1.5)
t939	1(1.5)

CHAPTER FIVE

DISCUSSION

5.1. PREVALENCE OF *S. AUREUS*

The data from this study showed that 22% of the recruited children at the Agogo Presbyterian hospital were colonized with *S. aureus* in the nasal cavity. It is estimated that about 30% of healthy human population asymptomatically carry *Staphylococcus aureus* in the nose and this acts as the most important source for infection transmission and auto-infection (Klutymans *et al*., 1997). *S. aureus* nasal carriage rates have been investigated in various populations of the world with few epidemiological studies being conducted in Ghana and several other African countries partly due to lack of adequate clinical microbiology infrastructure (Egyir *et al*., 2004a; Newman *et al*., 2011; Shittu *et al*., 2011). Most studies have investigated carriage in healthy children and school children with few studies investigating nasal colonization in the paediatric ward admission (Okwu *et al*., 2012).

The prevalence of nasal carriage of *S. aureus* from this study is higher than the 14% nasal carriage among inpatients reported by Egyir *et al*., (2013) but less than the nasal carriage of 23% among hospital staff as reported by the same study in Accra. Another study conducted by Egyir *et al*., (2014b) also reported a nasal carriage of 21% between two communities in Ghana. Although our study was among hospitalised children, the prevalence is similar to another study from Nigeria which documented a carriage rate of 18.3% for *S. aureus* among healthy primary school children (Okwu *et al*., 2012). Furthermore, our nasal carriage is lower than the 34.5% nasal carriage rate reported by Foday *et al*., (2010**)** among children between 1 and 25 months in Freetown, Sierra Leone. Younger children tend to have high carriage of *S. aureus* due to close contact with parents and relatives. However, the prevalence established in this study is higher than the 12.5% carriage rate found among 1- to 2- year age group reported by Ko *et al*., (2008) in Seoul, Korea. Kolawole *et al*., (2013) also reported a prevalence of 31.8% carriage of *S.*

aureus among 192 hospitalised patients tested. It is important to note that Kolawole *et al*., (2013) screened both the nose and the skin and that could be a reason for the high prevalence. Another study reported a prevalence of 20.89% nasal colonisation of *S. aureus* among patients admitted to a paediatric department in Italy (Gesualdo *et al*., 2014). In a study among hospitalised individuals in Bolivia Chao in Latin America, Bartoloni *et al*., (2014) also reported 14.6% nasal carriage of *S. aureus*. A report of a study among inpatients in nine hospitals in Switzerland however, produced a higher *S. aureus* nasal colonisation rate of 41.3% (Heininger *et al*., 2007). Sharif and co-workers in their study among 403 children admitted to Shahidbeheshti hospital in Iran also found that 49.1% were colonized with *S. aureus* in their nose (Sharif *et al*., 2013).

It has been noted that, *S. aureus* is a common cause of infections in Ghana (Newman *et al*., 2006). A study conducted by Tagoe *et al*. (2011) in south eastern part of Ghana reported that 57.6% of all the pathogens causing hospital acquired infections were *S. aureus*. Saana *et al*., (2013) also found 51.4% *S. aureus* among nose swabs from Kumasi. Though, clinically the discovery of this high presence of *S. aureus* in the anterior nares of these children does not indicate infection, it is believed that subsequent invasive infections may occur when the protective layer of the skin or mucosal barrier is breached thereby allowing the organism to have access to the adjoining tissues or the blood stream from these sites of carriage. It is an important risk factor for autoinfection and transmission of this pathogen within the hospital setting and the community (Boucher *et al.*, 2010). Even though Krebes *et al*., (2011) did not find enough evidence to support the claim that prior nasal carriage of *S. aureus* is a significant risk factor for acquisition of *S. aureus* on admission to a hospital, Von Eiff *et al*., (2001) and Wertheim *et al*., (2005) believed colonizing individuals may serve as reservoirs for subsequent infections and

transmission to other patients and that, elimination of nasal carriage reduces the incidence of *S. aureus* infections in some patients.

5.2. PREVALENCE OF MRSA

Among the 120 *S. aureus* isolated, two of them were found to be *mecA* positive and that represented 1.7%. This result is in agreement with the phenotypic screening which identified 2(1.7%) of the isolates to be cefoxitin resistant. The MRSA prevalence reported here is similar to the 1.3% nasal carriage of MRSA among the all the participants as reported by Egyir *et al.* (2013). The nasal carriage rate is however, less than the prevalence of 10% among *S. aureus* isolates from inpatients (Egyir *et al.* 2013). The low prevalence of MRSA recorded in this study is also similar to previous reports of 3% among *S. aureus* isolates (Egyir *et al*., 2014a) and 0.3% among nasal carriers between two communities in Accra (Egyir *et al*., 2014b). Similar prevalence of nasal carriage rates have also been reported in other countries. Heininger *et al*., (2007) found 0.18% MRSA nasal carriage among inpatients in 9 hospitals in Switzerland. MRSA nasal colonization prevalence of 1.15% among hospitalised children was reported by Gesualdo *et al*., (2014). The prevalence of MRSA in healthy school children in Nigeria was estimated to be 10.8% (Okwu *et al*., 2012). Kolewale *et al*., (2013) also documented 11.5% MRSA carriage among patients in a Nigerian hospital.

Sharif *et al*., (2013) found a high rate of 68.6% MRSA among children admitted to Shahidbeheshti hospital in Iran which they attributed it to the wide use of antibiotic in their center. Odonkor *et al*., (2012) also found 33.6% of clinical isolates to be MRSA in Accra. This however, does not represent nasal colonisation and therefore cannot be directly compared to the MRSA colonization rates in our study. More importantly, these MRSA isolates were not confirmed by molecular test as each test has it limitations. The origin of the isolates could also be a contributory factor to this high prevalence because

these clinical isolates might have been exposed to antibiotics during treatment making them to acquire resistance genes including *mec*A as compare to isolates from the nose. Although, this study has found low prevalence of MRSA similar to other reports, the tendency for transmission is possible and the accurate detection of MRSA is essential for the treatment of overt infections and the implementation of infection control practices. Though there are other methods for testing for MRSA like the oxacillin, cefoxitin test and PBP2a' agglutination test, the detection of the *mecA* gene or more recently the *mec*C gene have become the methods for confirmation of MRSA (Mathews *et al*., 2010 ; Monecke *et al*., 2013). Infections caused by MRSA strains have become one of the most common types of nosocomial infections, resulting in high morbidity, mortality, increased health care costs and length of hospital stay (Grundmann *et al*., 2006; Abdulager *et al*., 2015). MRSA nasal carriers may be at greater risk of developing MRSA infections as compared to methicillin-sensitive *S. aureus* (MSSA) carriers and non-carriers (Milstone *et al*., 2011). The risk of nosocomial transmission is likely to be high in hospital wards in developing countries, due to close physical proximity of patients, inadequate staffing, sanitizers and isolation facilities (Okeke *et al*., 2005). A growing concern is the appearance of CA-MRSA infections. CA-MRSA infections are associated with high morbidity and mortality partly due to the high rate of PVL that is associated with CA-MRSA. Also, for community-associated MRSA, transmission from person-to-person has been reported and several factors have been shown to predict disease (Boucher and Corey, 2008).

Therefore the recovery of MRSA in the nasal cavity of hospitalised individuals is a major concern because the chance of transmission in the hospitals and in the community is high. Precautions should be taken to reduce its spread in the hospital environment.

5.3. ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *S. AUREUS* STRAINS

The prevalence of resistance to antimicrobial agents was generally low for all the other antibiotics tested except for penicillin (96.7%), tetracycline (52.5%) and erythromycin (6.7%) which recorded high resistance among the *S. aureus* strains. This may be explained by the wide use of penicillin, tetracycline and erythromycin for treatment of bacterial infection in children. The high resistance pattern of *S. aureus* to penicillin and tetracycline in this study is similar to what have been reported from other studies in Ghana. Egyir *et al*., (2013) recorded 93% and 28% *S. aureus* resistance to penicillin and tetracycline respectively from nasal carriage isolates. According to Egyir *et al*., (2014a), resistance to antibiotics by isolates from nasal carriers was also below 5% for all antibiotics tested except for penicillin (91%) and tetracycline (25%). In a related study in Accra on clinical isolates, high resistance for erythromycin (6%), tetracycline (42%) and penicillin (97%) was also reported (Egyir *et al*., 2014b). The antimicrobial susceptibility pattern showed that all the MRSA strains were resistant to penicillin, cefoxitin and tetracycline.

The trend of antibiotic susceptibility of isolates from this study is similar to that observed from other studies in Africa where prevalence of resistance to tetracycline (28%–48%) and penicillin (86%–93%) have been reported (Breurec *et al*., 2011; Shittu *et al*., 2011). It is clear that the phenomenon of high resistance to tetracycline and penicillin is wide spread in Africa. The increased resistance to these commonly used antibiotics have been attributed to self-medication and indiscriminate use of these antibiotics to treat infections. It is easy to purchase these drugs over the counter without medical prescription (Newman *et al*., 2011).

All the isolates recorded 100% susceptible to teicoplanin and linezolid and this could be due to the relatively inaccessibility and unavailability of these antibiotics. This study

recorded nine antibiotypes: $P10^R$ $TE30^R$ (42.5%); P10R TE30R E15R (5%); P10, SXT25 (1.7%) and P10, TE30, FOX30 (1.7%). This could be due to cross-resistance among penicillins, tetracyclines and/or macrolides, sulphonamides. The clinical importance of this is that, penicillin and tetracycline should not constitute a first line therapeutic regimen in the treatment of *S. aureus* infections.

Based on the available data on antibiotic resistance, Newman and colleagues proposed a re-evaluation of the use of these antibiotics in the treatment of infection in Ghana due to the high levels of resistance observed against these antibiotics (Newman *et al*., 2011).

5.4. PREVALENCE OF PVL (*LUKS-PV* /*LUKF-PV*) AMONG *S. AUREUS* ISOLATES

In this study, 57.5% of the *S. aureus* isolated were found to be PVL positive. This is higher than the prevalence of 27% reported from nasal carriers in Ghana (Egyir *et al*., 2014b). A high PVL prevalence of 60% was also reported from clinical isolates from Ghana (Egyir *et al*., 2014a). The high rate of PVL positive *S. aureu* appears to be a characteristic genetic trait of *S. aureu* strains from Africa compared to Asia, USA and Europe isolates, where this virulence factor is rare (Schaumburg *et al*., 2014; Holmes *et al.*, 2005). Among the MRSA isolates, one was PVL positive and the other was negative for PVL. *S. aureus* harbouring PVL encoding genes have been associated with specific *S. aureus* infections such as SSTI, severe bone and joint infections and severe necrotising pneumonia. The high prevalence of PVL among the isolates is of special interest since PVL is a common feature of most CA-MRSA clones (Ruimy *et al*., 2008). The dissemination of *S. aureus* carrying PVL in the hospitals and communities in Ghana will have a serious problem on healthcare since the routine assessment of this toxin is usually not investigated by our clinical laboratories.

5.5. DIVERSITY OF ISOLATES ESTABLISHED BY *SPA* TYPING

The *spa* typing has established a high genetic diversity among the isolates as indicated by the recovery of 35 *spa* types among all the *S. aureus* tested. This study showed that the most widespread and predominant *spa* types among the *S. aureus* isolates were t355 (20%), t084 (14.9%) and t939 (10.7%). Studies in Ghana have also reported these *spa* types to be the most predominant types among *S. aureus* isolated from nasal carriers (Egyir *et al*., 2013; Egyir *et al*., 2014b). This present study has further confirmed the suggestion that these *S. aureus* types are well established and wide spread in the human population of Ghana. These *spa* types are also commonly found across several African countries (Ateba Ngoa *et al*., 2012; Schaumburg *et al*., 2014). Many of the *spa* types recorded in this study have also been reported in the Southern part of Ghana. The MRSA strains from this study belong to t1096 and t4454. The *spa* type t1096 was PVL positive and t4454 was negative for PVL. The two new *spa* types: t15727* and t15728* were both PVL negative. Except these *spa* types: t008, t021, t085, t1476, t1510, t157, t15727*, t15728*, t2147, t450, t537, t591, t6063, t616, t645, the rest of the *spa* types had at least a PVL positive isolate.

Molecular typing using *spa* typing has become the most widely method of typing *S. aureus*. The results obtained from typing can give useful diverse epidemiological information for local and global investigations **(**Strommenger *et al*., 2008). Even though MLST was not done in this study, it is worth mentioning it because the common *spa* types identified in this study have been linked to these sequence types (STs). These include: t355 (ST152), t314 (ST121) and t084 (ST15) which were also the most common sequence types found in a study conducted by Egyir *et al*., (2014a). The *S. aureus* strains t084 (ST15), PVL-positive ST152 (t355) and other PVL-positive MSSA lineages such as ST5, ST121, ST15 and ST30 have also been documented to be widely distributed in

African countries (Breurec *et al*., 2011; Shittu *et al*., 2011). These results indicate the wide distribution of different strains of *S. aureus* carrying PVL virulence encoding genes within hospitals and in the community. Therefore, the acquisition of the *mec*A by a PVL positive MSSA and the dissemination of PVL positive CA-MRSA could present a major problem in diseases management and infection control in a resource-limited country like Ghana where routine diagnostic does not assess toxins such as PVL.

5.6. DEMOGRAPHIC CHARACTERISTICS AND *S. AUREUS* CARRIAGE

In this study, more of the females were nasal carriers (23.8%) than the males (20.4%). This difference in nasal carriage was not however significant. Similar trend of 20.0% and 16.7% nasal colonisation among females and males respectively was reported by Okwu *et al*., (2012). This may suggest that females are more likely to be nasal carriers than males. However, there is no enough evidence in this study to back this claim. With regards to age, participants from the age group 6-10 years recorded the highest nasal carriage (36.1%) of *S. aureus* followed by the age group 1-5 years which had 20.6% nasal carriage. The age group 10-14 years had the least nasal carriage (15.0%). The age group 10-14 years are more likely to be hygienic than the younger ones that could account for the less number of positive nasal carriers. 22.0% of those from Agogo were nasal carriers while those from Bebuso and Pekyerekye had the highest nasal carriage of 30.8% and 30.0% respectively. Participants who were Ewe (33.2%) were more likely to be nasal carriers of the bacteria followed by the Northerners (23.8%). There was also a wide distribution of some specific *spa* types among the villages. *Spa* types: t355, t084, t939, t311 were widely distributed among the villages. The wide distribution of these specific strains in these villages could be attributed to the fact that these *spa* types are in wider circulation in Ghana (Egyir *et al*., 2014b).

5.7. STUDY LIMITATIONS

One limitation of this study is the inability to assess the nasal carriage of *S. aureus* and MRSA during admission and after discharge. This study did not compare the isolates obtained with isolates from infections in the hospital. Lastly, the study could not conduct comprehensive molecular strain typing of the MRSA that may provide more epidemiological information about them.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

This study presents the epidemiology of the prevalence of nasal carriage, antimicrobial susceptibility, virulence and the population diversity of *S. aureus* among hospitalized children. This study has shown that 22.0% of the children admitted to the Agogo Presbyterian Hospital were nasal carriers of *S. aureus* but with low (1.7%) prevalence of MRSA. Although nasal colonisation with *S. aureus* does not necessarily indicate *S. aureus* infection, screening is important as it allows the identification of potential sources

from which this organism can be acquired or transmitted to other individuals. The present study has also shown that, there is high resistance to penicillin and tetracycline by *S. aureus* with low or no resistance to the uncommon antibiotics. The study has also established a high prevalence (57.5%) of PVL-positive *S. aureus* among the isolates. The use of molecular typing in this study has established a high diversity of *S. aureus* strains (35 different *spa* types) and confirmed that these specific strains; t355, t084 and t939 are in wider circulation in Ghana. More importantly, this data established using genetic markers to characterize *S. aureus* strains in a health care setting has provided an overview of the genotypes of *S. aureus* carried among children on admission. Two new *spa* types were also discovered during the course of this study and they have been submitted to the *spa* database. This study therefore contributes and also enhances the current epidemiological knowledge of the population structure of *S. aureus* and MRSA in Ghana and can be used for local and global epidemiological studies.

6.2. RECOMMENDATION

There should be continuous surveillance of the nasal carriage of *S. aureus* and MRSA and the occurrence of *S. aureus* infections in this population.

Further studies should compare isolates from nasal carriage and hospital environment to see if there is transmission during admission

In the light of the high prevalence of PVL, routine virulence factors investigation in the clinical laboratory to identify toxin-associated diseases is recommended for the proper management of such diseases.

There is the need to reassess policies with regards to the use of penicillin and tetracycline within and outside the hospital environment due to the high resistance against them.

Further studies should compare admission and discharge rates and also use clinical information to determine if *S. aureus* plays a role in admission.

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APPENDIX

Appendix I. Preparation of media

A. Mannitol salt agar (Oxoid CM0085)

Formula g/L

Lab-lemco powder 1.0, Peptone 10, Mannitol 10, Sodium Chloride 75, Phenol red 0.025 and Agar 15.

111g was weighed and suspended in 1 litre of distilled water, dissolved completely by boiling and autoclaved at 121^0C for 15 minutes. It was cooled to about 45^0C and mixed well before pouring into sterile Petri dishes.

B. Columbia Blood Agar Base (Oxoid0331)

Approximate Formula g/ L

Pancreatic Digest of Casein 10.0, Peptone 5.0, Yeast Extract 5.0, Beef Heart Digest 3.0, Starch 1.0, Sodium Chloride 5.0 and Agar 15.0

To prepare blood agar, 39g of the agar base was weighed and suspended in 1 litre of distilled water, dissolved completely by boiling and autoclaved at 121^0C for 15 minutes. It was cooled to about 50^0C and 5% sterile defibrinated sheep blood added. It mixed well before pouring into sterile Petri dishes.

C. Mueller-Hinton Agar (Oxoid CM0337)

Formula g/L

Beef infusion 300.0

Casein hydrolysate 17.5

Starch 1.5

Agar 17.0

pH 7.2-7.4

To prepare this media, 35g of the agar base was dissolved in 1 litre of distilled water. It was then boiled shortly to dissolve the medium completely. It was sterilized by autoclaving at 121 ^{0}C for 15 minutes. It cooled and mixed well

before

pouring into

sterile petri

dishes

D. Brain heart infusion Broth (BD)

Typical Formula g/L `Lab-Lemco‟ powder 10.0 Peptone 10.0 Sodium chloride 5.0 pH 7.5 ± 0.2 containers and sterilised by autoclaving at 121^0C for 15 minutes.

25g was added to 1 litre of distilled water. It was the mixed well and sterilized by autoclaving at 121^0C for 15 minutes. It was750ml was distributed into 2ml and 750ml of glycerol added when saving a sample.

Appendix II. Procedure of biochemical test

Gram stain

Proceedure: A drop of normal saline was placed on a well labelled clean grease-free glass slide using a 1ul sterile inoculating loop; a colony of an overnight culture of the bacterial isolate was emulsified with the normal saline to make a smear. The smear was heat fixed on a slide heater. The slide was flooded with crystal violet (primary stain) for 60 seconds after which the stain was washed from the slide with slow running tap water. The smear was flooded with iodine (mordant) to fix the primary stain. The iodine was rinsed with water after 60 seconds**.** The slide was decolourised with acetone for 2-3 seconds and rinsed off almost immediately**.** The counter stain; safranin was added and left for 60 seconds before being rinsed off. The stained smear was air dried, and then observed under the microscope using X100 oil immersion objective lens of the microscope. Purple cocci appearance was indicative of staphylococcus.