CARRIAGE RATE OF *STAPHYLOCOCCUS AUREUS* AMONG HEALTH CARE WORKERS AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA

BY

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DECLARATION

I **Dr. Godwin Chakolwa** do declare that this dissertation represents my own original work and that it has never been done in Zambia before. I have not submitted this work for any other qualification at the University of Zambia or any other University.

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APPROVAL

This dissertation of Godwin Chakolwa has been approved in partial fulfilment of the requirements for the degree of Master of Science in Medical Microbiology at the University of Zambia.

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ABSTRACT

Staphylococcus aureus is a frequent cause of hospital-acquired infections worldwide. The carriage of *S. aureus* among healthcare workers has been associated with transmission of the bacterium to susceptible patients. The aim of this study was to determine the carriage rate, antimicrobial susceptibility profile and *spa* type of *S. aureus* among healthcare workers at a large tertiary referral hospital in Lusaka, Zambia. This was a prospectivecross-sectional study that involved the collection of nasal and hand swabs from 140 healthcare workers from May to July 2017 at the University Teaching Hospital in Lusaka. In this study, we recruited 103 nurses, 28 doctors and 9 laboratory scientists. Conventional microbiological methods were used to isolate and identify *S. aureus*. Antimicrobial susceptibility testing, including methicillin resistance was determined using the disc diffusion method, while molecular analysis of the isolates was achieved by *spa* typing. The overall *S. aureus* carriage among the health care workers was 17.1% (24/140).Of these, 13.6% (19/140) and 8.6% (12/140) were nasal and hand carriers, respectively. Carriage rate was highest among doctors (17.9%,5/28),followed by nurses (17.5%,18/103) and laboratory scientists (11.1%,1/9). About25.8% (8/31) of the *S. aureus* isolates were methicillin-resistant and showed resistance to more than four antibiotics. About 25.8% (8/31) of the *S. aureus* isolates were positive for the *spa* gene. Of these, seven were typeable. Two *spa* types, t015 (42.8%, 3/7) and t069 (14.3%, 1/7) were detected, whereas the *spa* type for 42.8% (3/7) of the isolates were unknown. The carriage of *S. aureus* and prevalence of Methicillin-resistant *S. aureus* among healthcare workers examined was high. This calls for regular intervention measures such as screening and decolonizing of healthcare workers to reduce the carriage and spread of this pathogen in healthcare centres in Zambia.

Key words: Antimicrobial resistance; Carriage rate; Healthcare workers; Methicillin-resistant *Staphylococcus aureus*; *spa* typing.

DEDICATION

This dissertation is dedicated to my wife Geddes N. Chakolwa and my daughter Faith Buleme Chakolwa.

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ABBREVIATIONS AND ACRONYMS

BURP	Based Upon Repeat Pattern
CA- MRSA	Community Acquired or Associated Methicillin Resistant *Staphylococcus aureus*
DNA	Deoxyribonucleic Acid
HA-MRSA	Hospital Acquired or Associated Methicillin Resistant *Staphylococcus aureus*
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Susceptible *Staphylococcus aureus*
MLST	Multilocus Sequence Typing
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
PFGE	Pulse-Field Gel Electrophoresis
RFLP	Restriction Fragment Length Polymorphism
SLST	Single-Locus Sequence Typing
UTH	University Teaching Hospital
MOH	Ministry of Health
SCC	Staphylococcal chromosome cassette
HCWs	Healthcare Workers

CHAPTER 1: INTRODUCTION

1.1 Background

Staphylococcus aureus is an opportunistic invasive pathogen of humans and a frequent cause of hospital acquired infections (Nicoletti et al., 2006, Lin et al., 2007). These infections are serious and often difficult to treat due to high rate of antibiotic resistance. The infections caused range from mild skin infections to life threatening conditions such as bacteremia, endocarditis and osteomyelitis (Nicoletti et al., 2006). Carriage of *S. aureus* among healthcare workers (HCWs) is a risk factor for transmission and development of these infections among patients in hospital settings (Diekema et al., 2001). In addition, carriage of the bacteria has also been known to be a risk factor for development of autoinfections among carriers (Stevens et al., 2010, Weidenmaier et al., 2012, Muthukrishnan et al., 2013). Furthermore, HCWs who are carriers provide a link through which cross transmission between community acquired (CA) and Hospital acquired (HA) *S. aureus* strains occurs (Conceicao et al., 2014a). This subsequently leads to increase in morbidity in the community. The transmission of the bacteria from colonized or infected HCWs occurs by direct contact or through fomites such as contaminated equipment or environment (Tammelin et al., 2003).

The emergence of virulent and multidrug-resistant strains has furthermore seriously increased morbidity and impeded effective treatment of *S. aureus* infections. Notable among these resistance mechanisms is methicillin resistance which is due to acquisition of the *mecA* gene by *S. aureus* strains. This gene codes for an alteration in the penicillin-binding protein (PBP) which leads to decreased affinity for *β*-lactam antibiotics (Harkins et al., 2017). The genetic variability has resulted in emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) that is difficult and expensive to treat (Ahmed et al., 2012, Whittington et al., 2017).

The knowledge on carriage rate among HCWs, genetic relatedness of *S. aureus* isolates and its antimicrobial resistance pattern provide the baseline data for infection control purposes. This study investigated the carriage of *S. aureus* carriage among health workers at the University Teaching Hospital in Lusaka.

1.2 Statement of the Problem

The increasing prevalence of *S. aureus* especially methicillin resistant strains among HCWs worldwide is a growing public health concern. Colonised HCWs act as a link in the transmission of the pathogen among patients and the community. However, there is a paucity of data on nasal and hand carriage, antimicrobial susceptibility patterns and genotypes of *S. aureus* among HCWs in Southern Africa including Zambia. Interestingly, laboratory records at UTH show relatively high isolation rates of *S. aureus* (about 30% and 34%) from burns and blood infections respectively (UTH laboratory records and Ministry of Health antibiogram guide 2015). Notably, 43% of these *S. aureus* isolates were methicillin resistant (Samutela et al., 2015). Furthermore, a study showed that 17.8% of white coats worn by HCWs at UTH are contaminated with *S. aureus* with 68% of the isolates being methicillin resistant (Mwamungule et al., 2015). However, the source of this *S. aureus* was not explored.

1.3 Justification of Study

Since infections caused by *S. aureus* result in increased hospital stay, costs and death, it is important to control the spread of this bacterium in hospital settings. Effective control measures depend on knowing the transmission dynamics, the genetic relatedness and an up-to-date

antimicrobial susceptibility profile of *S. aureus*. HCWs are more likely to be colonised than persons in the general population, presumably because of increased exposure. Therefore, screening hospital staff for nasal and hand carriage of *S. aureus* provides baseline data such as the carriage rates, genetic relatedness and antibiotic sensitivity patterns of the *S. aureus* upon which control measures such as decolonization can be based (Ahmed et al., 2012, Emaneini et al., 2017). Current antimicrobial susceptibility profiles are necessary for the selection of the appropriate empirical treatment for *S. aureus* infections and also for policy formulation (VanEperen and Segreti, 2016). This study was the first of its kind in Zambia to provide data on carriage rates, antimicrobial susceptibility profile and genotypes of *S. aureus* from nose and hands of HCWs.

1.4 Research Questions

What are the carriage rates, antimicrobial susceptibility profile and genetic relatedness of *S. aureus* from the nose and hands of health care workers at University Teaching Hospital in Lusaka?

1.5 Objectives

1.5.1 General Objective

To determine the carriage rate of *Staphylococcus aureus* among health care workers in different departments at the University Teaching Hospital in Lusaka from May to July 2017.

1.5.2 Specific Objectives

I. To detect *Staphylococcus aureus* from hands and nasal cavities of healthcare workers.

II. To determine the possible risk factors associated with carriage of *S. aureus.*

III. To determine the antimicrobial susceptibility patterns of the isolates.

IV. To determine the genetic relatedness of the *S. aureus* isolates.

CHAPTER 2: LITERATURE REVIEW

2.1 Carriage Sites for *Staphylococcus aureus*

S. aureus has been known to colonise several sites on the human body among which the anterior parts of the nostrils form the primary ecological niche (Rongpharpi et al., 2013). Carriage of bacteria in the anterior nostrils has been associated with seeding carriage in other extra nasal sites such as hands, pharynx, vagina, axillae, perineum, gastrointestinal tract and skin that is either intact or inflamed. However carriage of *S. aureus* in some extra nasal sites can still occur without colonisation of the anterior nares. In addition, carriage in some sites has been shown to be more prevalent than the anterior nostrils, for instance colonization of the throat by *S. aureus* is more prevalent than colonization of the anterior nostrils (Hamdan-Partida et al., 2010). However, owing to its importance as a reservoir for transmission, several studies to determine *S. aureus* carriage among healthcare workers worldwide have centred on nasal carriage (Khanal et al., 2015, Khatri et al., 2017, El Aila et al., 2017). Apart from that, nasal carriage has been known to pose a risk for autoinfection and contamination of equipment (Albrich and Harbarth, 2008, Muthukrishnan et al., 2013, Saadatian-Elahi, 2013) . Furthermore carriage of the bacteria on hands may indicate poor hygiene practices among healthcare workers (Lin et al., 2007, Visalachy et al., 2016). There is no reported difference in colonization sites between MRSA and *S. aureus* (Sollid et al., 2014). The carriage of *S. aureus* in these sites can be transient or persistent. Persistent carriage has been documented to be common in extranasal sites and in people with subclinical infections (Albrich and Harbarth, 2008). Furthermore persistent carriers are often colonised by a single strain of *S. aureus* unlike transient carriers. However both transient and persistently colonised healthcare workers play a key role in the transmission of *S. aureus* among patients and people in the community (Peacock et al., 2001).

2.2 *Staphylococcus aureus* Carriage Rates

Determination of carriage rates among HCWs in hospital settings is an important tool in curbing the spread of *S. aureus* especially MRSA among patients and the community. Carriage rates provide baseline data on which infection control measures can be based. This is because high carriage rates among HCWs have been associated with increased infections among patients in hospital environments. Variations in carriage rates have been observed among healthcare workers worldwide. Despite that, certain patterns of carriage have been observed across the globe. Notably, developing countries especially those from Africa have had higher rates compared to developed countries. For instance, the level of carriage for MRSA among health care workers Europe and the United States has been known to range from 1.1% to 5.4% and nursing staff were the most colonized with an average carriage rate of 6.9% (Dulon et al., 2014, Sassmannshausen et al., 2016a). In contrast, the average *S. aureus* carriage rate is 37.4% and that of MRSA ranges between 12% and 24.4% among healthcare workers in developing countries(Omuse et al., 2012, Ateba Ngoa et al., 2012, Ahmed et al., 2012, Shibabaw et al., 2013, Conceicao et al., 2014b, De Boeck et al., 2015, Sassmannshausen et al., 2016b).

Furthermore, carriage rates have been found to vary among different healthcare professions. Several studies have, had different findings in carriage rates among different cadres of healthcare workers worldwide. An international review study involving thirty-one research studies from developed countries found the nursing profession to be the most colonised (Dulon et al., 2014). Similarly several studies from Africa and Middle East have identified the nursing profession to be more colonised. A research study to determine the carriage rate among healthcare workers

(HCWs) at King Khalid University Hospital in Saudi Arabia identified nurses to be the most colonized (Al-Humaidan et al., 2015). In another research study conducted on HCWs at Dessie Referral Hospital in North East Ethiopia found 12.7% of HCWs to be nasal carriers of MRSA and nurses were the most colonized (21.2%) (Shibabaw et al., 2013). On the contrary, two research studies from India and Nepal found Doctors and laboratory workers respectively to be the most colonized than other HCWs (Shakya et al., 2010, Rongpharpi et al., 2013).

2.3 Risk Factors Associated with *S. aureus* Carriage

Several risk factors have been known world over to predispose HCWs to carriage of *S. aureus*. These factors include gender, age, smoking, duration of working years, antibiotic usage, chronic underlying conditions such as diabetes, profession, area of service and lack of adherence to hygiene conditions and infection prevention measures (Wang et al., 2009, Al-Humaidan et al., 2015, Legese et al., 2018). Presence of co-morbidities such as systemic and localised conditions increase the risk of colonisation with *S. aureus* (Pathare et al., 2016). Furthermore, departments within hospital settings predispose HCWs to colonisation with *S. aureus* carriage because of high health worker-patient contact frequency. Despite judicious antibiotic usage being curative for bacterial infections, it has been known to predispose users to Methicillin Resistant *Staphylococcus aureus* (MRSA) colonisation. The multidrug resistant nature of MRSA gives it an advantage over other antibiotic susceptible and protective bacteria to colonise the individual under medication. Additionally *S. aureus* develops antimicrobial resistance quickly thereby increasing the risk of antibiotic users to become carriers. Furthermore the male gender, advanced age, long duration of healthcare service, and nursing profession predispose to colonisation with *S. aureus.*

2.4 Antibiotic Susceptibility Patterns

The emergence of *S. aureus* strains that are resistant to many antimicrobial drugs is a global problem in hospital environments (Olufunmiso et al., 2017, Siddiqui et al., 2017). The increase in resistant strains has reduced treatment and control options for *S. aureus* infections in hospitals (Olufunmiso et al., 2017). Development of resistance to antimicrobials by *S. aureus* has been shown from recent studies to be on the rise worldwide (Diekema et al., 2001, Naimi et al., 2017). Notably resistance to Penicillin, Methicillin and Vancomycin respectively developed recently (Nicoletti et al., 2006, Shibabaw et al., 2013, Olufunmiso et al., 2017). The implications of this increase in resistance lie in the need for second–line antibacterial which are more expensive and need monitoring. The determination of antimicrobial resistance involves the use of molecular or phenotypic methods. The most widely used phenotypic method is the Kirby Bauer disc diffusion. This method involves the use of discs each coated with a different antimicrobials (Naimi et al., 2017). Various methods are used to detect resistance in *S. aureus* to methicillin, Other methods available for MRSA identification are PCR-based *mecA* detection, latex agglutination test for detection of penicillin binding protein (PBP2a), E-test, agar and broth dilution, quenching fluorescence assay and Chromogenic media (Hirvonen, 2014, Palavecino, 2014, Alipour et al., 2014, Singh et al., 2017).

2.4.1 Mechanisms of Antibiotic Resistance

Staphylococcus aureus has developed a wide range of mechanism of antimicrobial resistance against all the antimicrobial drugs that have been used in the treatment of staphylococcal infections. This is because of the ability of this bacterium to adapt quickly to antimicrobial

pressure. The emergence and spread of drug-resistant *S. aureus* has posed a major threat to public health. Recent studies have shown that lack of laboratory guided treatment of infections and the widespread use of antibiotics has caused selective pressures that have led to the development of these resistant strains (Harkins et al., 2017).

The major targets for antibiotics in *S. aureus* include the cell envelope, the ribosome and nucleic acids (Foster, 2017). Drugs that target the cell wall synthesis are main stay of therapy in *S. aureus* infections. Notable among these is penicillin, modified-penicillin (methicillin) and glycopeptides antimicrobial drugs. These antimicrobial drugs inhibit cell wall biosynthesis in bacteria and are important in the treatment of staphylococcal infections. The resistance of *S. aureus* to penicillin lies in the ability of the bacteria to produce an enzyme called beta-lactamase. This enzyme is a penicillinase that cleaves the *b*-lactam ring of the penicillin molecule resulting in a hydrolytic degradation product that has no inhibitory effects. The b-lactamase structural gene is carried on the transposon that is located on the plasmid or integrated in the chromosome.

Methicillin is modified penicillin that is able to resist degradation by beta-lactamase. Resistance to methicillin comes as a result of acquisition a gene (*mecA*) that encodes for the homologue of the penicillin binding protein (PBP2) called PBP2a with reduced affinity for B-lactam antibiotics. Resistance to methicillin is a predictor of resistance to all beta-lactam antibiotics. The *mecA* gene encoding methicillin resistance is carried on the staphylococcal cassette chromosome SCCmec element which is believed was horizontally acquired.

Glycopeptides such as vancomycin and teicoplanin are among the last therapeutic options available in the treatment of methicillin resistant *Staphylococcus aureus* (Foster, 2017). For this reason, prevention of *S. aureus* infections and emergence of resistant strains is critical.

Glycopeptides bind to the dipeptide D-Ala4-D-Ala5 of lipid II thereby preventing transglycosylation and transpeptidation catalysed by PBP2 and PBP2a and also antagonises peptidoglycan remodelling. It is feared that *S. aureus* will acquire vancomycin (*van*) resistance gene from vancomycin resistant enterococci (VRE) that will lead to serious invasive infections untreatable with vancomycin. The *van* genes code for inducible enzymes that take over the biosynthesis of peptidoglycan precursors leading to a lipid II molecule with D-lactate replacing D-Ala5. Sadly, resistance to Vancomycin and other glycopeptides is gradually being reported in sporadic places (Ahmed et al., 2012, Olufunmiso et al., 2017, Siddiqui et al., 2017).

2.5 Genetic Relatedness of *Staphylococcus aureus*

Molecular characterization of *S. aureus* is key to the quick identification of prevalent strains and provides the baseline data required for the control and prevention of *S. aureus* infections. Genotyping *S. aureus* is critical for studying of strain origin and clonal relatedness of circulating strains in hospital environments. *Spa* typing is a widely used molecular method in the determination of *Staphylococcus aureus* strains (Koreen et al., 2004). This molecular tool utilizes a short sequence of the repeat region of the *spa* gene which codes for surface protein (Protein A) that serves as virulence factor for *S. aureus.* The genotypes referred to as "*spa*-types", are based on highly variable Xr region sequences of the *spa*-gene that is used to classify *S. aureus* strains (Votintseva et al., 2014). The pattern of this *spa* gene varies from one geographical region to another (Asadollahi et al., 2018). For instance, the spa types t032, t008 and t002 are prevalent in Europe; t037 and t002 in Asia; t008, t002, and t242 in America; t037, t084, and t064 in Africa; and t020 in Australia (Ateba Ngoa et al., 2012, Samutela et al., 2017, Asadollahi et al., 2018). Apart from that certain *spa* types have been associated with methicillin resistance. Notably, all

the isolates related to *spa* type t032 in Europe were MRSA. Furthermore *spa* types t037 in Africa and t037and t437 in Australia were all methicillin resistant. A number of diverse *Spa* types have been identified among the MRSA clones in Africa. A recent study in Luanda identified t084 as the most frequent spa type. However, distribution of *Spa* types t042 and t044 appear to be limited to North African countries (Abdulgader et al., 2015). In Zambia *Spa* types t064, t2104, t355 and t1257 are prevalent among isolates from patients at the University Teaching Hospital (Samutela et al., 2017). However, data on *spa* types of *S. aureus* strains circulating HCWs at UTH is lacking.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Design

This study was a prospective cross-sectional study.

3.2 Study Site

The study was conducted for a period of six months at the University Teaching Hospital (UTH) in Lusaka. UTH is a referral hospital that is located in the capital city of Zambia. It is the largest healthcare institution in the country.

3.3 Study Population

The study population included health care workers from the surgery, medicine, paediatrics, obstetrics and gynaecology wards, neonatology, and laboratory (TB and Microbiology). Healthcare workers sampled included doctors, nurses, and laboratory scientists. Epidemiological data such sex, age, ward, years of health care service, and level of education, occupation, recent antibiotic use and risk factors such as smoking habits, nasal abnormalities and history of underlying diseases such as hypertension, chronic obstructive pulmonary disease and diabetes mellitus was collected from participants using a questionnaire that was issued prior to sample collection.

3.4 Inclusion Criteria

HCWs that come in direct contact with patients were included in the study at the University Teaching Hospital in Lusaka.

3.5 Exclusion Criteria

HCWs not involved in the provision of direct health care services such as office workers were not included in the study. Healthcare workers who do not work at UTH were excluded in the study.

3.6 Sample Size Calculation and Sampling Frame

In order to calculate the sample size it was assumed that 16.6% of HCWs in Zambia carry the bacteria in anterior nares and/or on their hands. This ensured a 5% allowable error and a 95% confidence level. Sample size was calculated using the formula for prevalence studies (Pourhoseingholi et al., 2013). The formula involves two stages; the calculation of sample size from an infinite population (>50,000) followed by calculation of final sample size from a finite population.

$$\mathbf{SS\ (n)} = \mathbf{Z^2\ \mathit{P}\ (1 - \mathit{P})\ /\ d^2}$$

Where: SS (n) = Sample size

Z = Z value (1.96 at 95 % confidence interval)

P = Expected prevalence. 16.6% (0.166) (from a previous study)

d = Precision (0.05).

Therefore:

$$n = 1.96^2 \times .166 \times (1 - .166) / 0.05^2$$

n = 213 HCWs

Therefore the number of HCWs that was to be included in the study was 213. However this sample size was not met as only 140 HCWs consented to participate in the study. These healthcare workers were distributed as shown in Table 3.6 below.

Table 3.1 Sampling Frame

Department	HCWs	Proportion
Medical Ward	22	15.7%
Laboratory	10	7.1%
Obstetrics and Gynaecology	24	17.1%
Surgery	49	35%
Paediatrics	23	16.4%
Medicine and Surgery	12	8.6%
Total	140	100%

3.7 Recruitment of Healthcare Workers

The matron in charge of the block was first informed about the study before going to the wards. In each ward the sister in charge was informed about the details before recruitment any HCW. The HCWs in each ward were first spoken to by principal investigator about the detail of the research. Those who agreed to participate were then given a written consent form to sign. During this process of data collection names and any identifiers were not used. Each participant was assigned a number upon being enrolled in the study. The details of the procedure, consent form and the questionnaire are attached in appendices A, B and C respectively.

3.8 Sample Collection

Samples were collected from healthcare workers present at the University Teaching Hospital from May to July 2017. By using pre-moistened sterile cotton swabs, specimens were collected

from the anterior nares and palms of the HCWs. The samples were collected by rotating the swabs gently for five times on both nares of the study participants so that the tip was entirely at the nasal osteum. Similarly, a second swab was used to swab both palms of the consenting healthcare worker. The samples were then transported in armies transport media and were inoculated onto Tryptone Soy broth within one hour of collection.

3.9 Identification of *Staphylococcus aureus*

Swabs were inoculated into Tryptone Soy broth for 18-24 hours and thereafter subcultured on blood agar. Colonies that were brown or white, beta-haemolytic and round characteristic of *S. aureus* were subcultured on Mannitol Salt Agar (MSA) for identification. These MSA plates were incubated at 37˚C for 24hrs. The yellowish appearance of colonies on MSA was indicative of positive fermentation of mannitol which was characteristic of *S. aureus*. Thereafter Gram staining and microscopy were done. Isolates that were gram positive, cocci shaped, arranged in pairs and clusters were identified as *S. aureus.* Confirmation of the suspected isolates was done using positive results from Catalase, Coagualase and DNAse tests (Winn and Koneman, 2006, Kateete et al., 2010). Methicillin susceptible *S. aureus* strains were differentiated from MRSA using Cefoxitin discs on Mueller Hinton Agar (CLSI, 2015).

3.9.1 Gram Staining

Crystal violet was added on a previously air dried and heat fixed smear on a glass slide of the suspected *S. aureus* colonies. The slide was then washed with distilled water followed by addition Lugol's iodine for 1 minute washed off using distilled water. The decolourisation was

done by addition of acetone for approximately 10-15 seconds and immediately washing with distilled water. The slide was then counterstained with dilute carbolfuchsin for about 30 seconds and rinsed thoroughly with water. The slides were thereafter air dried and then examined using a light microscope at x100 under oil immersion (Winn and Koneman, 2006). Observation of purple cocci shaped bacteria arranged in clusters was characteristic of *S. aureus.*

3.9.2 Catalase Test

Catalase test was performed with 3% hydrogen peroxide (Winn and Koneman, 2006). Using a sterilised wire loop a single colony was picked and emulsified into the drop of hydrogen peroxide onto the glass slide. Formation of bubbles indicated a Catalase positive test result. *S. aureus* is Catalase positive. This test differentiated Staphylococci from Streptococci.

3.9.3 Coagulase test

For tube coagulase tests, colonies of gram positive, cocci-shaped and Catalase positive test isolates were suspended in 1 ml of citrated sheep plasma in sterile glass test-tubes (Winn and Koneman, 2006). A Positive control tubes was added using a DNase producing control strain (*S. aureus* ATCC 25923) (Kateete et al., 2010). The test tubes were incubated at 37ºC and observed for coagulation (formation of clots) for 3 - 4 hours. If there was no coagulation after 4 hours the tube incubation was extended up to 24 hours. Coagulation denoted a positive result. *Staphylococcus aureus* is coagulase positive and this differentiates it from other *Staphylococcus species.*

3.9.4 DNase Test

The DNase test was performed on Deoxyribonuclease (DNase) media plates. DNase is an enzyme produced by *S. aureus* that cleaves DNA. The DNA present in the agar is hydrolysed by DNase if this enzyme is produced by the organism. After incubation of the DNA agar plate, the plate was flooded with an excess of 15ml of 1N hydrochlololoric acid (HCl), which precipitated any unhydrolysed DNA and produced cloudiness (Winn and Koneman, 2006, Kateete et al., 2010). A zone of clearance was visible where the DNA has been hydrolysed. For isolates were tested for DNase production per DNA plate by drawing horizontal on the agar plate, creating small squares. A control strain ATCC *Staphylococcus aureus* 25923, was included on every plate as a positive control. Each square on the plate was inoculated with a single colony from pure culture of each isolate. The plate was then incubated under aerobic conditions overnight at 37°C for 18-24hrs. After incubation the plate was flooded with 1M HCl and left for a minute after which excess HCl was discarded. A zone of clearance around the inoculum was read as a positive result.

3.10 DNA Extraction

Mixed glycerol stocks of *S. aureus* cultures were prepared by suspending several loopfuls of bacteria taken by sweeping across the Mannitol salt agar plate in 1.5ml of saline (E and O laboratories) with 200ul of 45% glycerol for storage at -80 centigrade. Taking a sweep across the plate rather than picking a single colony for glycerol stocks which allowed us to maintain the genetic diversity of nasal strains in the samples for later analyses.

Crude *S. aureus* DNA extracts ('boilates') used for *spa*-typing were made from mixed glycerol stocks that were revived on blood agar plates. Using a 1mm loop, a small amount of bacteria was emulsified into 60ul of Tris-EDTA (TE) buffer (Sigma-Aldrich), then heated in thermocycler at 99.9 centigrade for 10 minutes and centrifuged at 13,200 rpm for two minutes. About 40ul of supernatant were removed without disturbing the pellet and stored at -20 centigrade for use as a PCR template (Votintseva et al., 2014)

3.10.1 DNA Amplification

The *spa* gene was amplified by PCR using the primers 1095F (5' AgACgATCCTTCggTgAgC-3') and 1517R (5'-gCTTTTgCAATgTCATTTACTg-3') (Votintseva *et al.*, 2014, Dag *et al*., 2003). For amplification of the *Staphylococcus* protein A (*spa*) repeat region, a PCR is performed in a total volume of 50 µl containing cleaned DNA, 200 µM deoxynucleoside triphosphates (dATP, dCTP, dGTP,and dTTP), 10 pmol of each primer, 5 µl of 10-fold concentrated PCR Buffer II (Applied Biosystems), MgCl2 1.5 mM, and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems). The cycle conditions used were as follows: initial denaturation at 95°C for 4 minutes, followed by 30 cycles of 95°C for 30 seconds (denaturation), 60°C for 30 seconds (annealing) and 72°C for 45seconds (extension). The final extension time of 10 minutes at 72°C was used. Electrophoresis of 5µl of the PCR product was performed on a TrisBorate-EDTA (TBE) agarose gel (wt/vol) (100V) containing 1µl ethidium bromide (10 mg/ml). A 500bp ladder (Thermo Scientific, Hanover, MD, USA) was used as a molecular weight standard and all gels were visualised using a Biotop Biosens SC -645 Gel Documentation System (Biotech Co. Ltd, Shanghai, China)

3.10.2 DNA Sequencing

The PCR product is purified by an enzymatic method using exonuclease I (New England Biolabs GmbH, Frankfurt-Hoechst, Germany) and shrimp alkaline phosphatase (Amersham Pharmacia Biotech). Briefly, 5µl of the PCR product is incubated with 1 U of each enzyme at 37°C for 30 minutes. Then the enzymes are inactivated at 80°C for 15 minutes and the PCR products finally stored at 4°C. The amplicons are sequenced using the ABI Prism Big Dye Terminator v 3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). The sequencing reaction requires 1.0 µl of premix from the kit, 1.5 µl TrisHCl/MgCl2 buffer (400mM Tris-HCl; 10mM MgCl2), 10 pmol of sequencing primer, and 2 µl of the cleaned PCR product in a total volume of 10 µl. The same primers used in the PCR are used for sequencing with an annealing temperature of 60°C. All sequencing reactions are performed using a T1 Thermocycler (Whatman Biometra, Göttingen, Germany) with 25 cycles of denaturation (96°C, 10 s), and extension (60°C, 4 min). The sequencing products are purified using the Centri-Sep Spin Columns (Princeton Separations, Adelphia, NJ) and are prepared for running on the ABI 3100 Avant Genetic Analyzer in accordance with the instructions of the manufacturer (Applied Biosystems)

3.11 Antimicrobial Susceptibility Tests

The inoculum was prepared by making a saline suspension of isolated colonies selected from an 18-24 hours Mannitol salt agar plate and the suspension was adjusted to match the 0.5 McFarland turbidity standards by using saline and the vortex mixer. A sterile cotton swab was dipped into the adjusted suspension and excess inoculum was removed by pressing the swab firmly on the inside wall of the tube. The dried surface of a Mueller Hinton agar plate was inoculated by streaking the swab over the entire surface. The antimicrobial discs were placed firmly on the surface of the inoculated agar plate using sterile forceps. The plate was then incubated at 35 ^{o}C within 15 minutes after application of the discs. After 18 hrs of incubation, the plates were examined and the diameters of the zones of inhibition were measured. Results were defined as susceptible, intermediate, or resistant, according to the approved guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015).

3.12 Data Collection Tool

A pretested structured questionnaire was used to collect epidemiological data needed for evaluation of *S. aureus* carriage and practices of HCWs. Data included demographics, length of service, present and past health history, and recent use of antibiotics, profession, ward and level of education.

3.13 Data Analysis

Statistical analysis of categorical variables such as susceptibility patterns was performed using Chi-square or Fisher's exact test, when the expected frequency in a cell is less than 5. *S. aureus* carriage was the dependent variable. Factors that predispose to carriage of *S. aureus* were the

independent variable. Logistic regression was used to determine the association between carriage of *S. aureus* and the factors that predispose to carriage. Blasting and editing of sequences was done using Ridom TraceEdit. Determination of the *spa* types was done using the centre for Genomic Epidemiology (Bartels et al., 2014). All the statistical tests were performed at 5% level of significance. Most of the data obtained in the study were descriptive and thus were presented in frequency tables and figures.

CHAPTER 4: RESULTS

Our study set out to determine the carriage rate of *Staphylococcus aureus* among healthcare workers and the risk factors associated with carriage of the bacteria at University Teaching Hospital. The study also sought to determine antibiotic susceptibility profile and the genetic relatedness of the *S .aureus* isolates. The following were the findings.

4.1 Risk Factors, Socio-demographic characteristics and Departments included in the study

Out of the 140 HCWs screened for *S. aureus* carriage, 107 (76.4%) were females and 33 (23.6%) were males. Fifty six (40%) were below 30 years and 84 (60%) were above 30 years old. Of these 28 (20%) were doctors, 9 (6.4%) were laboratory scientists and 103 (73.6%) were nurses as shown in the following Table 4.1.

Table 4.1 : Risk Factors, Socio-demographic characteristics and Departments included in the study

Variable	*S. aureus* n (%)		P value (*P<0.05*)
	Present	Absent	
Department			0.477
Paediatrics	3 (13.04%)	20 (86.9%)	
Obstetrics and Gynaecology	5 (20.83%)	19 (79.17%)	
Medicine and Surgery	2 (16.67%)	10 (83.33%)	
Surgical Ward	9 (18.37%)	40 (81.63%)	
Medical Ward	4 (18.18)%	18 (81.81%)	
Laboratory (TB & Bacteriology)	1 (10.00%)	9 (90.00%)	
Age			0.847
> 30	13 (15.48%)	71 (84.52%)	
< 30	11 (19.64%)	45 (80.36%)	
Years of Service			
< 10	17 (16.83%)	84 (83.17%)	0.303
>10	7 (17.94%)	32 (82.05%)	
Gender			
Male	6 (18.18%)	27 (81.81%)	0.501
Female	18(16.82%)	89 (83.18%)	
Profession			0.886
Doctor	5 (17.86%)	23 (82.14%)	
Nurses	18 (17.48%)	85 (82.52%)	
Laboratory Scientist	1 (11.11%)	8 (88.89%)	
Antibiotic Use			0.695
Yes	8 (15.69%)	43 (84.31%)	
No	16 (17.98%)	73 (82.02%)	
Chronic Debilitating Condition			0.294
Present	5 (22.73%)	17 (77.27%)	
Absent	19 (16.10%)	99 (83.89%)	

4.2 Determination of *S. aureus* and MRSA Carriage among the HCWs

4.2.1 Proportion of Nasal and Hand carriage of *S. aureus* and MRSA among HCWs

Of the 140 HCWs that were screened for *S. aureus* carriage 13.6 % (19/140) were nasal carriers while 8.6% (12/140) HCWs carried *S. aureus* on the hands. Those who carried it in both the hand and the nose were 5.0% (7/140). Hence the overall carriage rate for *S. aureus* was 24/140 (17.1%). The overall MRSA carriage rate was 5.7% (8/140). The proportion of nasal and hand carriers of MRSA was 2.1% (3/140) and 3.6% (5/140) respectively. No HCW had both hand and nasal MRSA carriage. Of the 24 carriers 6 (18.18%) were males and 18(16.82%) were females. These findings are shown in Table 4.2.1 below.

Table 4.2 : Nasal and Hand Carriage Rates of *S. aureus* and MRSA

Variable		Hand n (%)	Nasal n (%)	Hand & Nose n (%)	Overall n (%)
***S. aureus* (Overall)**	**Present**	12 (8.6)	19 (13.6)	7 (5.0)	24 (17.1)
	Absent	128 (91.4)	121 (86.4)	133 (95.0)	116 (82.9)
MRSA	**Present**	5 (3.6)	3 (2.1)	0 (0)	8 (5.7)
	Absent	135 (96.4)	137 (97.1)	140 (100)	132 (94.3)

Results indicate the higher prevalence of MRSA among *S. aureus* isolates from hands than those from the nasal cavity

4.2.2 Carriage Rates of *S. aureus* and MRSA Carriage among the Different Cadres of HCWs

The carriage for S. *aureus* and MRSA among doctors was 17.9% and 8.7% respectively. The rate of isolation of *S. aureus* and MRSA among nurses was 17.5% and 5.8% respectively. The laboratory scientists had *S. aureus* carriage of 11.1% for *S. aureus* and did not carry any MRSA as shown in Figure 4.1 below.

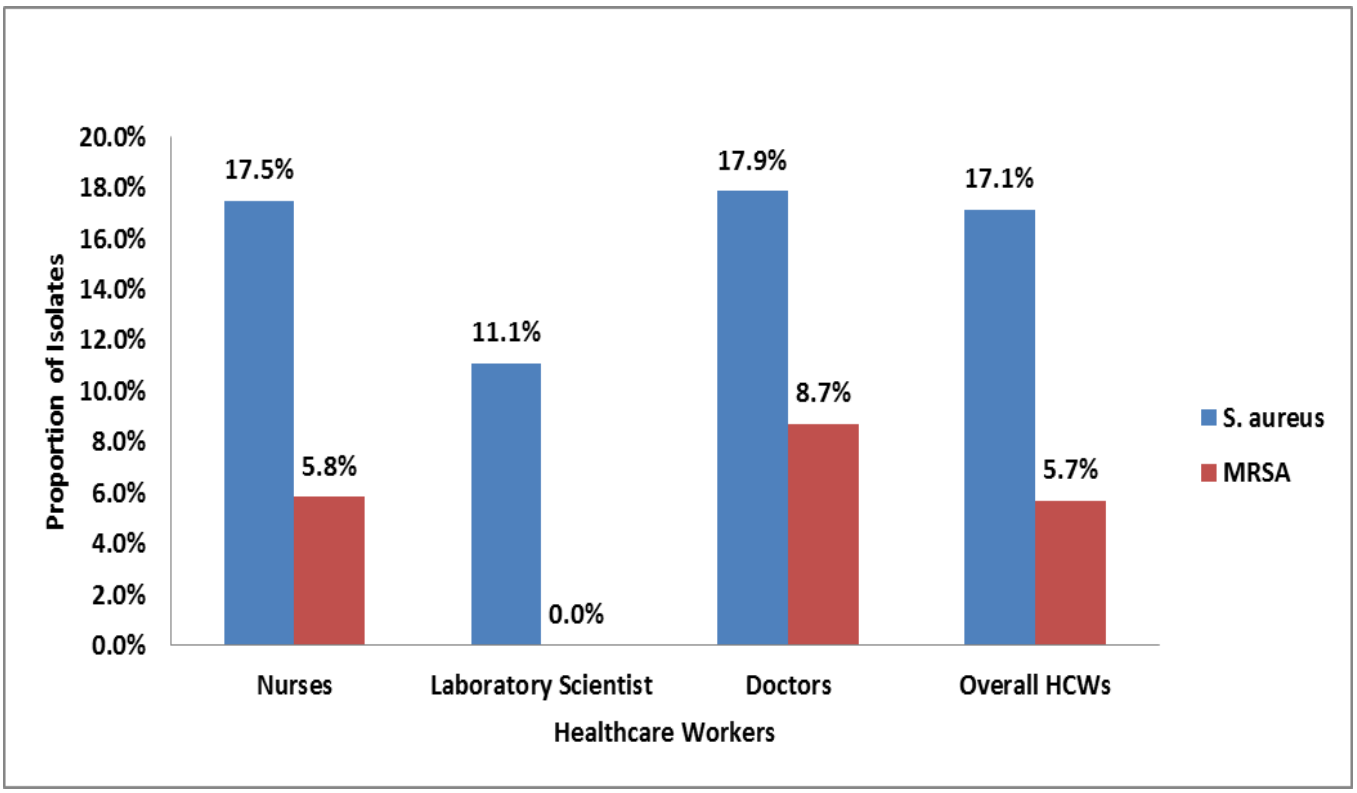

Figure 4.1 : Carriage Rates of *S. aureus* and MRSA among the Different Cadre of HCWs

The results showed that Doctors had the highest carriage rate compared to other professions.

4.2.3 Carriage Rates of *S. aureus* among the Different Hospital Departments

Carriage of *S. aureus* was highest among HCWs from the department of gynaecology followed by surgery and medicine respectively as shown in figure 4.2

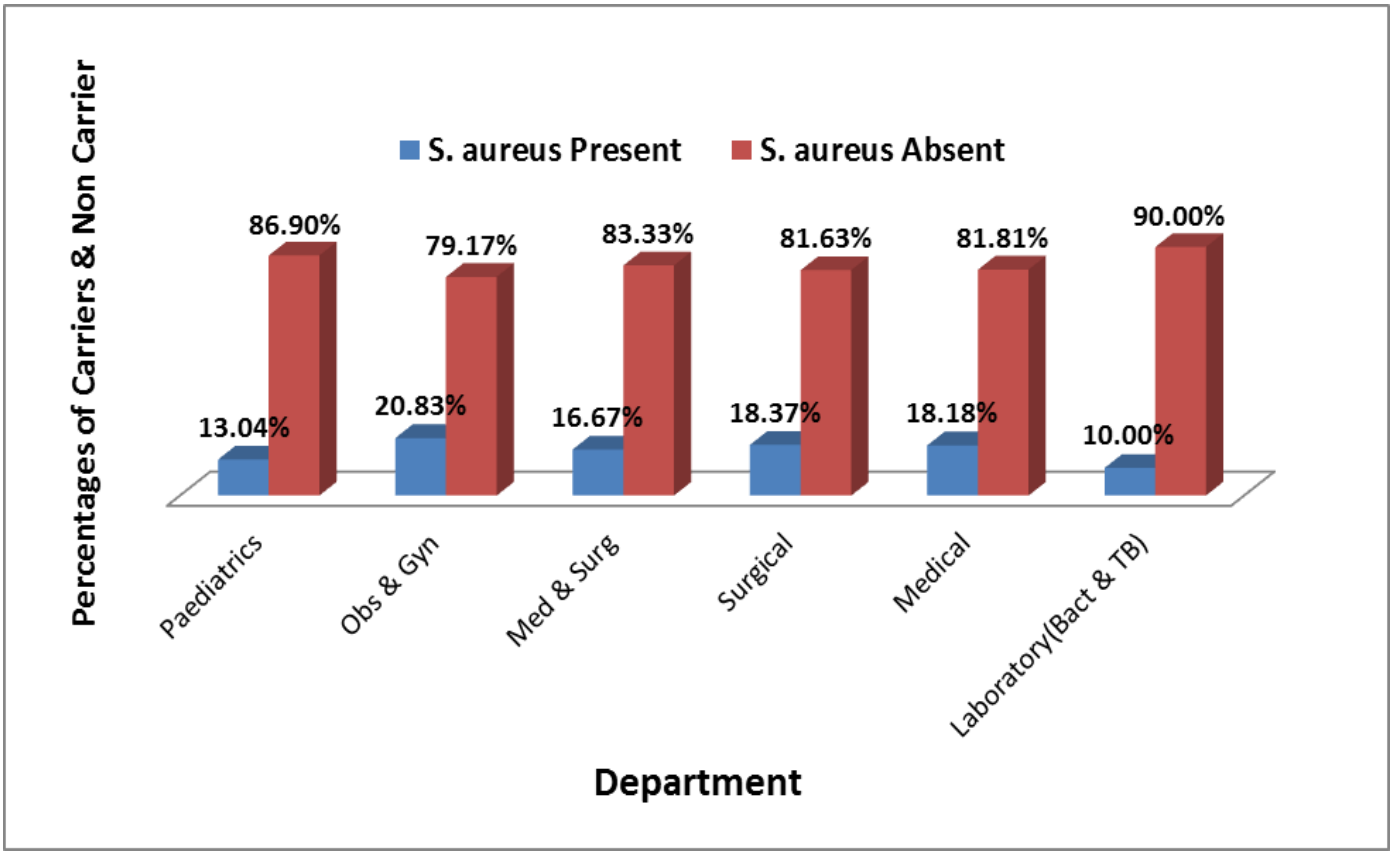

Figure 4.2: Carriage rates of *S. aureus* According to Different Hospital Departments

The obstetrics and gynaecology department had highest carriage rate and Laboratory department had the lowest as shown in fig 4.2.

4.3 Determination of the Antimicrobial Susceptibility Patterns of the Isolates

The antibiotic susceptibility of the *S. aureus* isolated was determined using various antibiotics as shown in Figure 4.3a. Amikacin and teicoplanin had the highest sensitivity at 100% while penicillin and ampicillin had the lowest at 22.6% and 19.4% respectively. The overall cefoxitin (methicillin) resistance was 25.8%. The proportion of MRSA isolates among *S. aureus* isolates from the nose and the hands were 15.8% and 41.7% respectively.

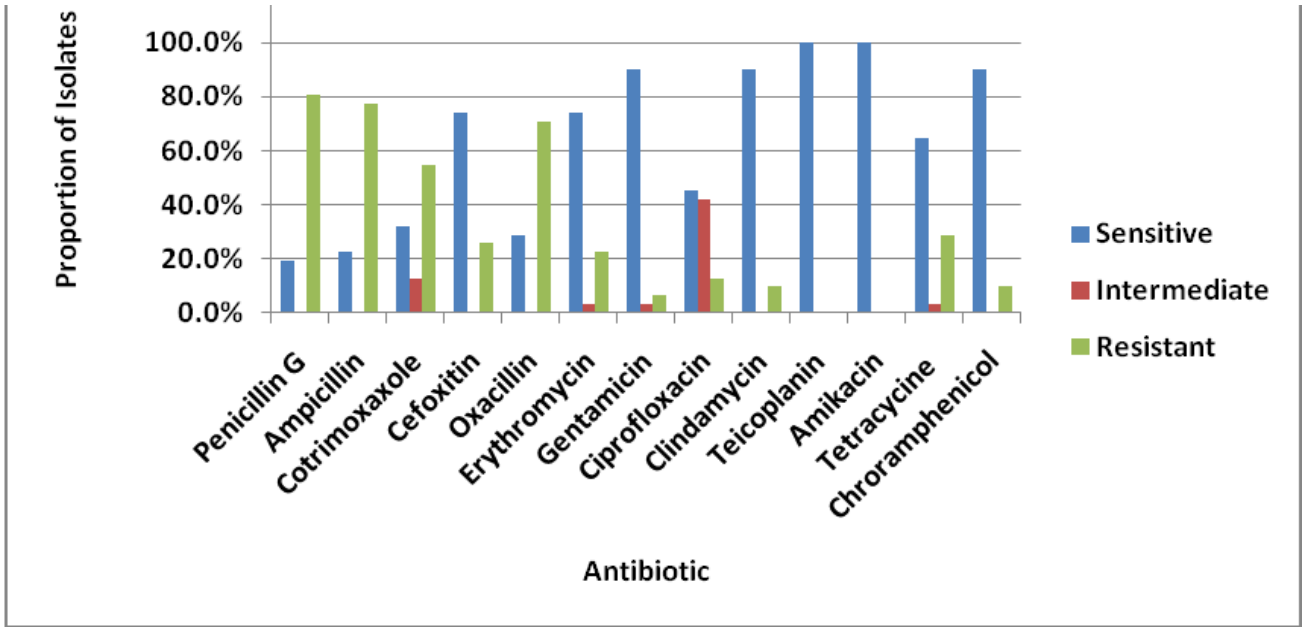

Figure 4.3a: Antibiotic susceptibility of the *S. aureus* isolated

The results show high resistance by *S. aureus* isolates to first-line antibiotics such as penicllin and ampicillin. Notably, the isolates exhibited low resistance to teicoplanin and amikacin.

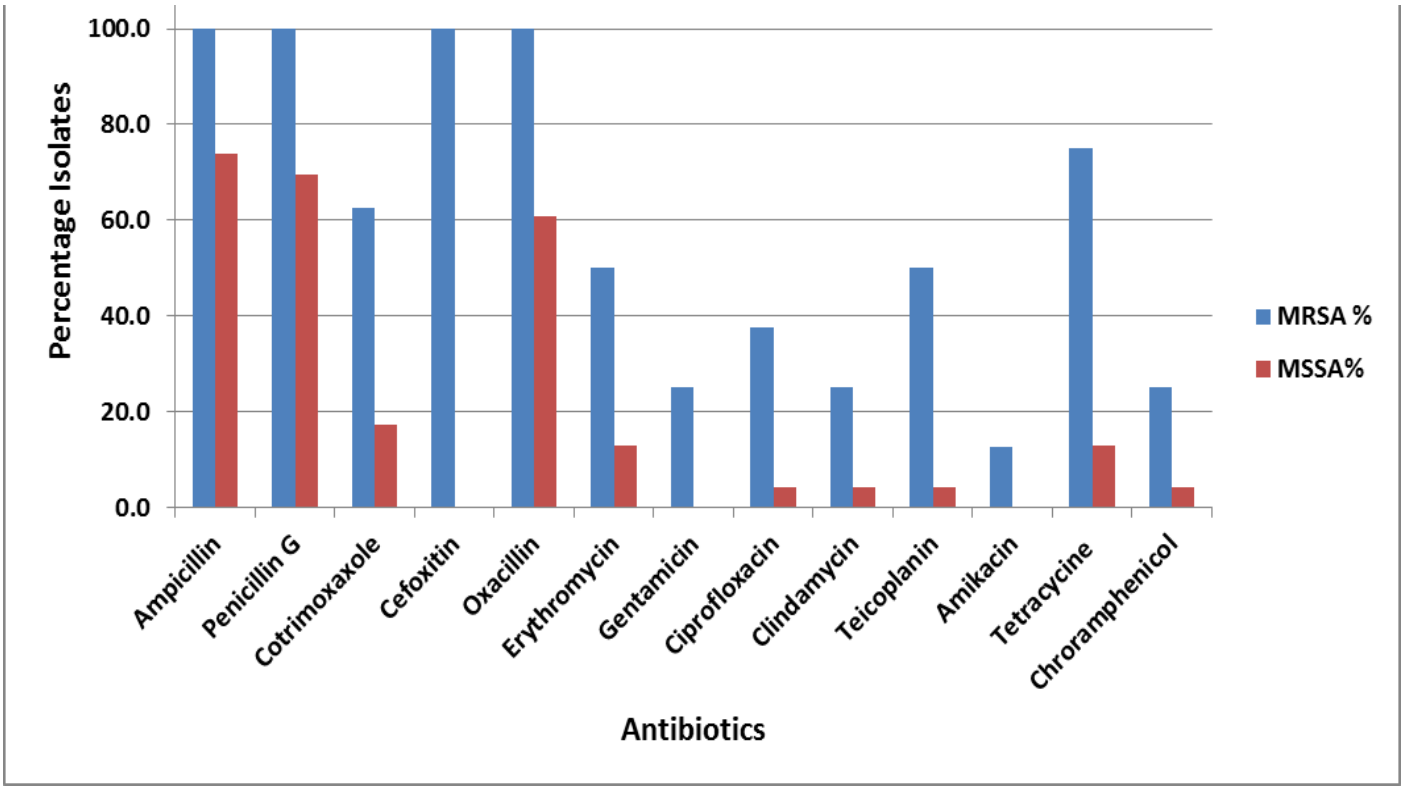

Figure 4.3b: Differences in resistance patterns between MRSA and MSSA isolates

Methicillin Resistant *S. aureus* (MRSA) isolates showed higher resistance to antibiotics compared to methicillin susceptible *S. aureus* (MSSA) isolates (figure 4.3b). Notably MRSA exhibited 100% resistance to ampicillin and penicillin.

Table 4.3 Antibiotic resistance patterns of the MRSA isolates (n=8)

Resistance Pattern	Proportion of Isolates %(n)
AMP+PG +TS + Gen + Ery + Cip +C + T +CD	12.5% (1)
AMP+PG + TS + Gen + Ery + C + T	12.5% (1)
AMP+PG + TS+ Ery + Cip	12.5% (1)
AMP+PG + Ery + Gen + Cip+ C + T	12.5% (1)
AMP+PG+ Ery + C	12.5% (1)
AMP+PG + TS + Te	25% (1)
AMP+PG + Te	12.5% (1)

Abbreviations: AMP, Ampicillin; PG, Penicillin; TS, Co-trimoxazole; Gen, Gentamicin; Ery, Erythromycin; CD, Clindamycin; Cip, Ciprofloxacin; C, Chloramphenicol; Te, Tetracycline

These results show multidrug resistance pattern of MRSA isolates to commonly used antibiotics at UTH.

4.4 Determination of Staphylococcal protein A (*Spa*) Types

Twenty-six percent (8/31) of the *S. aureus* isolates were positive for *spa* gene after PCR as shown Figure 4.4a below.

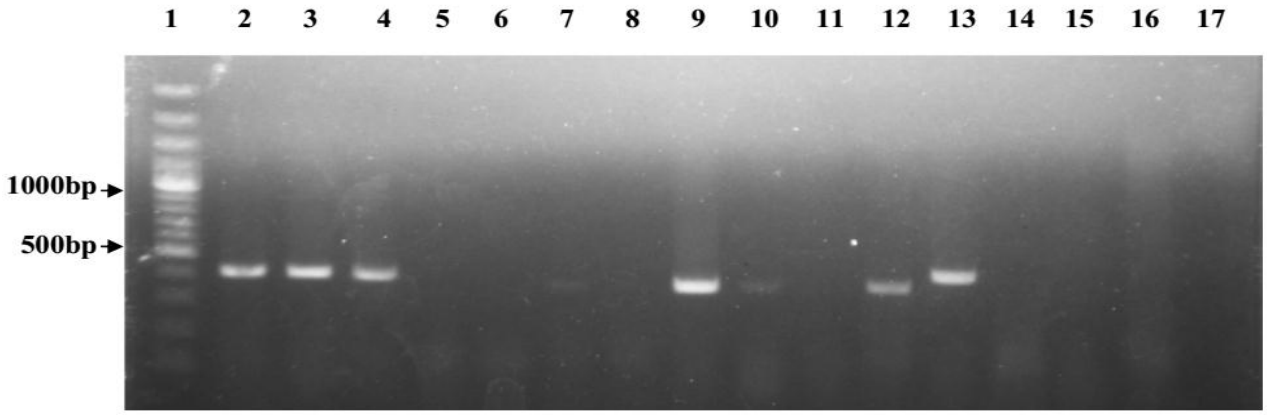

Figure 4.4a: Representative *spa* typing gel picture: Lane 2, 3,4,12 and 13 were positive while the rest of the lanes were negative for the *spa* gene.

A. 2018-06-22_E08_001_F1

Staphylococcus aureus strain CFSAN007835 chromosome, complete genome
Sequence ID: CP017685.1Length: 2676924Number of Matches: 2

Score	Expect	Identities	Gaps	Strand
577 bits(312)	4e-163	320/324(99%)	0/324(0%)	Plus/Minus

```
Query  1      CGATGCTCAAGCACCAAAAGAGGAAGACAACAACAAGCCTGGTAAAGAAGACGGCAACAA  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83552  CGATGCTCAAGCACCAAAAGAGGAAGACAACAACAAGCCTGGTAAAGAAGACGGCAACAA  83493

Query  61     ACCTGGTaaagaagacaacaaaaaacctggcaaagaagacggcaacaaacctggtaaaga  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83492  ACCTGGTAAAGAAGACAACAAAAAACCTGGCAAAGAAGACGGCAACAAACCTGGTAAAGA  83433

Query  121    agacaacaaaaaacctggtaaagaagacaacaacaaacctggtaaagaAGACGGCAACAA  180
              |||||||||||||||||||||||||||||||| ||||||||||||||||||  ||||||
Sbjct  83432  AGACAACAAAAAACCTGGTAAAGAAGACAACAAAAAACCTGGTAAAGAAGACAACAACAA  83373

Query  181    GCCTGGTaaagaagacaacaaaaaacctggtaaagaagacggcaacaaacctggtaaaga  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83372  ACCTGGTAAAGAAGACAACAAAAAACCTGGTAAAGAAGACGGCAACAAACCTGGTAAAGA  83313

Query  241    agacaacaaaaaacctggtaaagAAGACGGCAACGGAGTACATGTCGTTAAACCTGGTGA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  83312  AGACAACAAAAAACCTGGTAAAGAAGACGGCAACGGAGTACATGTCGTTAAACCTGGTGA  83253

Query  301    TACAGTAAATGACATTGCAAAAGC  324
              ||||||||||||||||||||||||
Sbjct  83252  TACAGTAAATGACATTGCAAAAGC  83229
```

B. 2018-06-22_A09_001_F5

Staphylococcus aureus partial spa gene for immunoglobulin G binding protein A precursor, strain IH76167, allele 11
Sequence ID: AM076298.1Length: 1378Number of Matches: 2

Score	Expect	Identities	Gaps	Strand
599 bits(324)	9e-170	324/324(100%)	0/324(0%)	Plus/Plus

```
Query  1      CGATGCTCAAGCACCAAAAGAGGAAGACAACAACAAGCCTGGTAAAGAAGACGGCAACAA  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  882    CGATGCTCAAGCACCAAAAGAGGAAGACAACAACAAGCCTGGTAAAGAAGACGGCAACAA  941

Query  61     ACCTGGTaaagaagacaacaaaaaacctggcaaagaagacggcaacaaacctggtaaaga  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  942    ACCTGGTAAAGAAGACAACAAAAAACCTGGCAAAGAAGACGGCAACAAACCTGGTAAAGA  1001

Query  121    agacaacaaaaaacctggtaaagaagacaacaacaaacctggtaaagaAGACGGCAACAA  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1002   AGACAACAAAAAACCTGGTAAAGAAGACAACAACAAACCTGGTAAAGAAGACGGCAACAA  1061

Query  181    GCCTGGTaaagaagacaacaaaaaacctggtaaagaagacggcaacaaacctggtaaaga  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1062   GCCTGGTAAAGAAGACAACAAAAAACCTGGTAAAGAAGACGGCAACAAACCTGGTAAAGA  1121

Query  241    agacaacaaaaaacctggtaaagAAGACGGCAACGGAGTACATGTCGTTAAACCTGGTGA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1122   AGACAACAAAAAACCTGGTAAAGAAGACGGCAACGGAGTACATGTCGTTAAACCTGGTGA  1181

Query  301    TACAGTAAATGACATTGCAAAAGC  324
              ||||||||||||||||||||||||
Sbjct  1182   TACAGTAAATGACATTGCAAAAGC  1205
```

Figure 4.4b: DNA sequences and blasts of the *spa* gene positive representative *S. aureus* isolates. The results showed that the obtained sequences belonged to *Staphylococcus aureus.*

The *S. aureus* isolates were found to be of two *spa* types as shown in the following Table 4.4. *Spa* type t015 was the most prevalent. Three isolates had unknown spa types.

Table 4.4 *Spa* types and their Distribution among *S. aureus* Isolates

Proportion of Isolates % (n)	Spa type	Repeat Succession	Position
37.5 (3)	t015	08-16-02-16-34-13-17-34-16-34	62-339
12.5 (1)	t069	08-16-02-16-34-13-17-34-16-16-34	62-363
37.5 (3)	Unknown		
12.5 (1)	Unsequenced		

CHAPTER 5: DISCUSSION

Carriage of *S. aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA) among healthcare workers has been documented to be a risk factor for transmission and development of staphylococcal infections among patients in healthcare centres (Siddiqui and Whitten, 2018). Owing to this fact, determination of carriage rates of *S. aureus* especially methicillin resistant strains among healthcare workers has been known to be a useful tool in the prevention of infections caused by this bacterium (Hawkins et al., 2011). In our study the overall carriage rate for *S. aureus* and MRSA among HCWs were 17.1% and 5.7% respectively. This carriage among HCWs is a source of concern as it would lead to high morbidity and mortality among patients, increased hospital stay and costs associated with treatment (Al-Talib et al., 2013). Our findings compares with results from a similar study that was done in Northern Ethiopia at Adigrat and Wukro Hospitals were the carriage rate for *S. aureus* and MRSA were 12% and 5.8% respectively(Legese et al., 2018). However our carriage rate for *S. aureus* was higher than that of a similar study that was done in Kisangani, the Democratic Republic of Congo where the carriage rate of *S. aureus* was 16.6% (De Boeck et al., 2015) but much lower compared to results reported from similar studies in Ethiopia (28.8%), Gabon (29%) and Principe (23.7%) (Ateba Ngoa et al., 2012, Shibabaw et al., 2013, Conceicao et al., 2014b). The differences in carriage rates of *S. aureus* and MRSA can be attributed to variations in sampling techniques, microbiological procedures and local infection standards (Khanal et al., 2015). The MRSA carriage rate of 5.7% obtained in the study was within the internationally recognised range of MRSA carriage (4.6% to 17.8%) among HCWs in non-outbreak settings(Albrich and Harbarth, 2008, Shakya et al., 2010, Dulon et al., 2014, Sassmannshausen et al., 2016b). However this MRSA prevalence is much lower compared to those obtained in similar studies that have been

reported in other African countries where carriage rate was above 10% (Ahmed et al., 2012, Shibabaw et al., 2013, Gebreyesus et al., 2013, Hefzy et al., 2016) but higher than findings from Kenya zero prevalence of MRSA was reported (Omuse et al., 2012).

The rates of MRSA isolation among *S. aureus* isolates has been found to differ from the region. In this present study the rate of isolation of MRSA among *S. aureus* isolates was 25% (8/32). This rate was lower compared to a study that was done in Northern Ethiopia 48.3% (14/29) but higher than those that were reported in Kisangani, DRC 15.9% (10/63) and Kenya 0% (0/45) (Omuse et al., 2012, De Boeck et al., 2015, Legese et al., 2018). The variations could be as a result of differences in microbiological procedures and adherence to infection control procedures among HCWs (Khanal et al., 2015).

Carriage has been found to vary among different profession of HCWs around the world. Our study found carriage to be highest among doctors, followed by nurses and the least was among the laboratory personnel (8.7%, 5.8%, and 0.0%). Similar findings were reported from studies in Assam where the carriage rates among doctors and nurses were 25% and 22.86% respectively (Rongpharpi et al., 2013). In contrast, a similar study in Saudi Arabia found carriage rates among nurses, doctors and laboratory technicians to be 23%, 22% and 19% respectively and an international review study found nurses to be more colonised than doctors (6.9% vs 4.0%) (Dulon et al., 2014, Al-Humaidan et al., 2015). The reasons for variation in carriage rates in our study could be due to low doctor to patient ratio compared to nurses at the University Teaching Hospital in Lusaka.

The gynaecology, surgery and medical wards were found to be high risk areas for colonisation with *S. aureus* (20.3%, 18.4% and 18.2%). These results compare with findings from a similar study in Assam and Nepal where departments of surgery and gynaecology were found to be high risk areas for *S. aureus* colonisation (Rongpharpi et al., 2013, Khanal et al., 2015). This could be due to high healthcare worker-patient contact that has resulted from very low HCW to patient ratio at UTH. HCWs above 30 years of age had a higher carriage rate at 19.64% than those who were below 30 years at 15.48%. This could have been due to the differences in duration of healthcare service provision. Male HCWs had a slightly higher carriage, at 18.2% compared to their female counterparts at 16.8%. Similar findings were reported from Nepal, India and Saudi Arabia (Shakya et al., 2010, Rongpharpi et al., 2013, Al-Humaidan et al., 2015). On the contrary a similar study in Ethiopia found females to be more colonised than male HCWs (Gebreyesus et al., 2013). This reason for high carriage among male HCWs at UTH could be poor adherence to infection prevention measures.

The carriage rate was higher among HCWs that worked for more than 10 years (17.9%) compared to those worked for less than 10 years (16.8%). Our findings compare with those of a similar study in Saudi Arabia (Al-Humaidan et al., 2015). Antibiotics usage three months prior to participation in the research study was protective against colonisation with *S. aureus.* This finding was not in agreement with findings of similar studies from Taiwan and Malaysia which found antibiotic usage to be a risk factor for colonisation with Methicillin Resistant *S. aureus* (Wang et al., 2009, Al-Talib et al., 2013). The difference could be attributed to variations in duration of antibiotic usage among participants. Similarly carriage was higher among HCWs that had chronic debilitating conditions like diabetes compared to those who did not (22.73%vs

16.10%). This finding was in agreement with that from Ethiopia which found diabetes to be a risk factor for colonisation with *S. aureus* (Legese et al., 2018). However, none of these risk factors assessed in our study were found to be significantly associated with the carriage rate of *S. aureus* (*P Value < 0.05*). These findings compare with those from a similar study in Saudi Arabia which found that all the risk factors assessed not to be significantly associated with Carriage rate of *S. aureus* and MRSA (Al-Humaidan et al., 2015).

Determination of antimicrobial resistance is an important tool in the successful treatment of bacterial infections and prevention of outbreaks in hospital settings. In our study *S. aureus* isolates showed high resistance against Penicillin G (80.7%) and Ampicillin (77.4%). This is a serious source of concern because penicillin and ampicillin are among the first line drugs that are used in the treatment of staphylococcal infection. The pattern of resistance to penicillin obtained in our study was comparable with results from similar studies from Zambia, Ethiopia, and Gaza Strip but was lower than those from Pakistan (Farzana et al., 2008). The reason for high rate of resistance to these common drugs could be due to the misuse of these antibiotics by HCWs and cross contamination with resistant strains from patients and the hospital environment. However, the antibiotic susceptibility profile of *S. aureus* isolates exhibited highest sensitivity to Amikacin (100%), Teicoplanin (100%), Chloramphenicol (90.3%), and Gentamicin (80.7%). This pattern sensitivity to Amikacin was in agreement with findings from a similar study in Nepal (100%) and Iran (87%) but was higher than the one observed in Pakistan (77%) (Farzana et al., 2008, Khatri et al., 2017, Pourakbari et al., 2017). These findings were also in agreement with those from a previous study in Zambia that detected 100% susceptibility of all MRSA isolates to Amikacin and Teicoplanin (Samutela et al., 2015). The variations in susceptibility of isolates

could be due to differences in usage of the Amikacin in the treatment of bacterial infections. The pattern of resistance to Teicoplanin was consistent with findings from Kenya (97%) and India (100%) (Singh et al., 2017, Gitau et al., 2018). The reason for the low resistance to Teicoplanin in our study could be due to the low usage glycopeptides at UTH for treatment of staphylococcal infections.

The overall methicillin resistance was 25.8%. The proportion of MRSA isolates from the nose and the hands were 15.8% and 41.7% respectively. All the MRSA isolates were resistant to more than five (5) antibiotics. Furthermore all MRSA isolates exhibited 100% resistance to Ampicillin, and Penicillin G. The pattern of resistance was in agreement with those from similar studies from Gaza Strip, Ethiopia, Saudi Arabia and Zambia (Shibabaw et al., 2014, Al-Humaidan et al., 2015, Samutela et al., 2015, El Aila et al., 2017).

Spa typing when combined is an important tool that is used in the epidemiological study of *S. aureus.* When combined with analysis of based upon repeat pattern (BURP), *spa* typing can be used to assign isolates into particular clonal lineages useful for national and international surveillance of *S. aureus* (Strommenger et al., 2008). In our study two 25.8% (8/31) *S. aureus* isolates were positive for the *spa* gene. The reason for the low rate of detection could be due to the absence of the *spa* gene in some isolates and the use of phenotypic methods during the identification of *S. aureus* (Baum et al., 2009). Two *spa* types (t015 and t069) were determined and three were unknown. Of the two, *spa* type t015 was the most prevalent (42.8%). The two *spa* types had similar repeat succession suggesting that the isolates were related and clonally disseminated. *Spa* type t015 was also isolated in Germany where it was detected in patients with

cystic fibrosis where it was designated as MLST sequence type ST45 (Garbacz et al., 2018). A study in Austria also detected *spa* type t015 where it was associated with graft infections in patients (Konstantiniuk et al., 2016). In another study at a Polish Neonatal Intensive Care Unit (NICU), *spa* type t015 was associated with infections in newborns and were found to be positive for *SCCmec* type IV among MRSA isolates (Romaniszyn et al., 2015). In another study from Europe both *spa* types t015 and t069 were associated with multiclonal colonisation and infection at a nursing home (Matussek et al., 2011). In a study from Nigeria *spa* type t069 was the most prevalent *spa* type among *S. aureus* isolates from poultry and was associated with the occurrence of staphylococcal enterotoxin genes (Ayeni et al., 2018). There is no much information about the spa type t069 compared to t015. A previous study in Zambia identified *spa* types t2104, t064, t1257, t355 and one unknown from *S. aureus* isolates from patients. *Spa* type t064 was the most prevalent and t355 was a singleton and most *spa* types were known to be hospital acquired and clonally disseminated (Samutela et al., 2017). These *spa* types from this previous study appear not to be related with the *spa* types that were found in our study. This could be due to differences in sources of isolates and times the two studies were conducted. The unknown *spa* types could not be compared with the known *spa* types because no repeat succession for unknown spa types were provided by the link we used to determine the *spa* types (Bartels et al., 2014).

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusions

Taken together, these results suggest that the carriage rate of *S. aureus* and MRSA among HCWs, as well as the prevalence of MDR-MRSA at UTH was high. These findings, along with the detection of spa type t015 and t069, which have been associated with clinical cases and multi-clonal colonisation, underscore the need to strengthen surveillance, infection prevention and control measures at UTH in order to prevent transmission of such strains from HCWs to patients.

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6.2 Recommendations

1. There is need for regular screening of healthcare workers for carriage of *S. aureus* and MRSA and decolonization of carriers.
2. There is need to strengthen prevention and infection control measures at UTH by incorporating the frequent training of HCWs on infection prevention measures.
3. There is also a need to monitor resistance pattern of *S. aureus* to all antibiotics that are used in the treatment of staphylococcal infections.
4. The study recommends laboratory guided use of antibiotics in the treatment of bacterial infections among patients and healthcare workers.
5. There is need for continued monitoring of the strains of *S. aureus* that are circulating at UTH by periodically determining their genetic relatedness and variability.

6.3 Limitations of the Study

Our study could not establish the genetic relatedness between the *S. aureus* strains circulating among healthcare workers with those causing infections among patients. This information would have been useful in understanding the epidemiology of *S. aureus* infections at the University Teaching Hospital.

6.4 Future Directions

There will be need to compare strains circulating among HCWs workers and patients by collecting samples from both HCWs and patients at the same time. There will be need to screen healthcare workers from other departments that were not included in this study so as to have the full picture about the strains of *S. aureus* that are circulating at the University Teaching Hospital.

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APPENDICES

Appendix A: Information Sheet

Part I: Information Sheet

This Informed Consent Form is for healthcare workers at the University Teaching Hospital who are involved in provision of direct health care services to patients. The title of the research is Carriage rate of *Staphylococcus aureus* among healthcare workers at the University Teaching Hospital in Lusaka, Zambia.

Introduction

I am Dr. Godwin Chakolwa doing a master's degree in medical microbiology at the University of Zambia. I am doing a research on the carriage of a bacterium called *Staphylococcus aureus* on hands, and in nasal cavities of healthcare workers at University Teaching Hospital in Lusaka. I am basically seeking to determine the carriage rate, genetic relatedness and antibiotic susceptibility profile of the bacteria. This study will be conducted through collection of swabs from nasal cavities and hands of the participating healthcare worker.

S. aureus is a frequent cause of localized and life threatening systemic infections such as bacteremia, osteomyelitis and endocarditis. The carriage of *S. aureus* among healthcare workers poses a risk to the people they come in contact with and also to themselves. This research will provide baseline data on carriage rate, strains of the bacteria and resistance patterns upon which transmission and infection control measures can be based. This study will be carried out in strict confidence and you will not be required to give your name. You will also be at liberty to answer all questions but should there be any questions that you feel uncomfortable with, you will be under no obligation to answer. The process of obtaining the samples is safe and will not cause

you any pain. However you may experience some discomfort that is associated with obtaining the nasal swabs.

All healthcare workers who work at the University Teaching Hospital in Lusaka and come in contact with patients are eligible to participate in the research. Your participation in this research is entirely voluntary. It is your choice whether to participate or not. No punishment will be meted for choosing not to participate in this research. You may change your mind later and stop participating even if you agreed earlier. You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected. The research study will take 6 months in total. You will be sampled only once during the research. The results of the study may not benefit you directly but they might benefit the future management of nosocomial infections. However, the results will be made known to you and if found to carry *Staphylococcus aureus* you will be referred to a physician for decolonization and treatment free of charge. The study will also enhance infection, prevention and control practices within the hospital and facilitate education of health care workers in this aspect.

We will not be sharing the identity of those participating in the research. The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key.

The knowledge that we get from doing this research will be shared with you through meetings before it is made widely available to the public. Confidential information will not be shared. After these meetings, we will publish the results in order that other interested people may learn from our research.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

1. The chairperson for the University of Zambia Biomedical Research Ethics Committee (UNZABREC) on Telephone: 260-1-256067, Telegrams: UNZA, LUSAKA, Telex: UNZALU ZA 44370, Fax: + 260-1-250753 or E-mail: unzarec@zamtel.zm or Ridgeway Campus. P.O. Box 50110 Lusaka, Zambia.
2. Dr. Godwin Chakolwa the principle investigator on mobile number 0979402674, or email gchakolwa@yahoo.com, of Plot no. L/12725/M, Off Kasama Road, Lusaka.
3. Dr. Gina Mulundu on 0977788804, School of Medicine, Pathology and Microbiology Department
4. Dr. Geoffrey Kwenda on 0979428815, University of Zambia, School of Medicine, Department of Biomedical Sciences.
5. Dr. Chileshe Lukwesa, on 0976403286, University Teaching Hospital, Pathology and Microbiology Department P/Bag RW 1X.

Appendix B: Consent Form

Title of Research Study**: Carriage rate of *Staphylococcus aureus* among healthcare workers at the University Teaching Hospital in Lusaka, Zambia.**

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Swabs will be collected from their nose and hand.
2. All the information throughout the research will be kept confidential
3. That the participant can withdraw from the study without jeopardizing his/her job or career.

I confirm that participants will be free to skip questions that they may deem personal or otherwise and they will be free to withdraw from the study any time without suffering any penalty.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Appendix C: Questionnaire

The purpose of this question is to obtain information for study purposes only. All information will be kept strictly confidential. This information will be used for the analysis and evaluation of the risk factors associated with *S. aureus* carriage. The information will go a long way in the implementation of infection control practices. The questionnaire consists of two sections A and B. Please take your time and complete it carefully and thoroughly, and then review it to be certain you have not left anything out.

If you have questions or concerns, we will help you with clarifications. We realize that some parts of the form will be unclear to you. Feel free to ask about any unclear part before, during or after filling in the form.

As a participant you are free to skip questions that you may deem personal or otherwise and you are free to withdraw from the study without any penalty.

Participant:

Ward/Department _____

Assigned #_____

Signature:_____

Section A: Demographic Data

1. Gender:

Male □

Female □

2. Age in years

Less than 30 □

30-50 □

More than 50 □

3. Education:

Secondary School Education □

Tertiary Education □

Others…….

4. Occupation:

Nurse □

Doctor □

Pharmacist □

Clinic Officer □

Laboratory Scientist □

Others……

5. Years of Working

0-9 □

10-19 □

20-36 □

6. Ward/Department

Laboratory □

Paediatrics □

Obstetrics and Gynaecology □

Neonatology □

Surgery □

Internal Medicine □

Others…….

Section B: Possible Risk Factors

1. Previous Hospitalization in past three months

Absent □

Present □

2. Antibiotic use in previous 3 months

Present □

Absent □

3. Smoking

Present □

Absent □

4. Nasal Abnormalities

Present □

Absent □

5. Underlying Disease

Diabetes Mellitus

Absent □

Present □

Chronic Obstructive Pulmonary Disease (COPD)

Absent □

Present □

Hypertension

Absent □

Present □

6. Do you use gloves when handling patients?

Yes □

No □

Sometimes □

7. Do you change gloves between patients?

Yes □

No □

Sometimes □

8. Cleaning hands after patient handling

	Always	**Sometimes**	**Rarely**
With water only			
With Soap and water			
With sanitizer			

9. How often do you wash your coats/ aprons/ uniforms?

Every day□

After two days□

After three days□

Appendix D: Ethics Clearance

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
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Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

19th October, 2016.

Our Ref: 005-08-16.

Dr. Godwin Chakolwa,
University of Zambia,
School of Medicine,
Department of Pathology and Microbiology,
P.O Box 50110,
Lusaka.

Dear Dr. Chakolwa,

RE: RESUBMITTED RESEARCH PROPOSAL: **"CARRIAGE RATE OF STAPHYLOCOCCUS AUREUS AMONG HEALTH CARE WORKERS AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA" (REF. No. 005-08-16)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 13th October, 2016. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr. S.H Nzala
VICE-CHAIRPERSON

Date of approval: 19th October, 2016. **Date of expiry:** 18th October, 2017.