NASAL CARRIAGE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF METHICILLIN RESISTANT *Staphylococcus aureus* AMONG HEALTH WORKERS AT *KAMPALA* INTERNATIONAL UNIVERSITY TEACHING HOSPITAL IN BUSHENYI, UGANDA

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A RESEARCH DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY OF KAMPALA INTERNATIONAL UNIVERSITY

JUNE, 2018

DECLARATION

I, **Abimana B. Justus,** declare that this research dissertation is my original work and has never been presented before for any award at any University or Institution of higher learning. Where ideas were borrowed the authors have been cited.

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DEDICATION

To my Mum Irene Ntirugurirwa and to my son Johnson Mukiza

ACKNOWLEDGEMENT

I am indebted to my supervisors Dr. Joel Bazira and Dr. Kato Charles Drago for tireless effort in guidance they offered me right from proposal writing up to generation of this report. I thank the administration of Kampla International University Teaching Hospital, especially the Executive Director for Hospital services; Prof. Robinson Sebuwufu for having allowed me carryout the study in the Hospital. Credit also goes to Faculty of medicine MUST through the Mbarara University research and training initiative project for having enabled me to acquire skills in molecular diagnostics which I used in this study. Acknowledgement goes to the contribution of Dr. Henry Sembuya for the technical advice and data management. The word of appreciation goes to the laboratory technicians whose help was indispensable in this study: Mr. Pius Theophilus and Mr.Michael Gumisiriza; Kampala International University Teaching Hospital laboratory, Mr. Ibrahim Ntulume and Mr. Jackson Muhwezi of Microbiology Department KIU Western Campus, Mr. James Mwesigye of Mbarara University of Science and Technology; Microbiology laboratory, Dr. Sylvester Ochwo and Mr. Christian Ndekezi of molecular biology laboratory of College of Veterinary medicine, Animal Resources and Biosecurity (CoVAB).

I cannot fail to thank all the Health care providers at KIU TH who participated in this study; without their cooperation this study would be futile.

Lastly but not the least, thank you Microbiology and Immunology department through the head, Ms Sarah Onkoba for giving me time and supported me to undertake this study.

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LIST OF ABBREVIATIONS

ATCC-	American Type Culture Collection
CA- MRSA-	Community Associated Methicillin Resistant *Staphylococcus aureus*
CLSI-	Clinical and Laboratory Standards Institute.
DNA-	Deoxyribo Nucleic Acid
FNBP-	Fibronectin binding proteins
HA-MRSA-	Hospital Associated Methicillin Resistant *Staphylococcus aureus*
HCWs-	Health Care Workers
IREC-	Institutional Research and Ethics Committee
KIU-	Kampala International University
MHA-	Muller Hinton Agar
M-PCR	Multiplex Polymerase Chain Reaction
MRSA	Methicilin Resistant *Staphylococcus aureus*
MSA-	Manitol Salt Agar
MSCRAMMs-	Microbial Surface Components Recognizing Adhesive Matrix Molecules
ORF-	Open Reading Frame
PBP-	Penicillin Binding Proteins
PBP2a-	Refractory Penicillin Binding Proteins
PPE-	Personal Protective Equipment
PVL-	Panton Valentine Leukocidin
SCCmec-	Staphylococcal Cassette Chromosome mecc

ABSTRACT

Background:

Methicillin Resistant *Staphylococcus aureus* is commonly encountered and it's a threat to health care services because of its ability to resist other antibiotic classes in addition to beta lactams. Health Care Workers are important reservoirs of *Staphylococcus aureus* and yet there is insufficient literature on their carriage rates of Methicillin Resistant strains. The purpose of this study was to determine the prevalence of nasal carriage of *Staphylococcus aureus* and to compare antimicrobial susceptibility pattern of Methicillin Resistant and susceptible *Staphylococcus aureus* isolates from health care workers of Kampala International University Teaching Hospital, south Western Uganda.

Materials and Methods:

A cross sectional study involving the culturing of nasal swabs from Health Care Workers at Kampala International University Teaching Hospital was carried out. Phenotypic and genotypic screening MRSA from isolated *Staphylococcus aureus* was done using cefoxitin disc and mecA gene amplification respectively. Antimicrobial susceptibility pattern of the MRSA and MSSA isolates was performed using Kiby_Bauer disc diffusion method.

Results:

Out of the 97 participants, 28(28.8%) were nasal carriers of *Staphylococcus aureus* of which 13 (46.4%) were phenotypically MRSA (resistant to cefoxitin) and only 8 (28.6%) were genotypically MRSA (had mecA gene). Methicillin resistant *Staphylococcus aureus* (both phenotypic and genotypic) isolates were all resistant to beta lactam drugs but susceptible to lincosamides, glycopeptides and aminoglycosides.

Conclusion:

The nasal carriage rate of *Staphylococcus aureus* and Methicillin resistant strains is high among health care workers and mecA gene is not the only genetic basis for resistance to methicilin drugs. Methicillin resistant strains showed higher resistance rate to commonly used antibiotics than methicillin susceptible strains.

Recommendations:

Future studies should consider whole genome sequencing to identify other genetic markers coding for methicillin resistance.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Staphylococcus aureus is a common bacterium found on the skin surface of people especially in the axilla, perineum and in the upper respiratory tract but also with ability to survive on inanimate objects (Lowy, 1998; Nester, Anderson, Roberts, Pearsall, & Nester, 2004; Schaechter, Medoff, & Eisenstein, 1993). The organism is known to cause a number of diseases ranging from uncomplicated skin and soft tissue infections to invasive serious infections, such as pneumonia, endocarditis, and sepsis (Lowy, 1998; Shibabaw, Abebe, & Mihret, 2013). Immune competent people maybe carriers with no symptoms but immune compromised people develop serious complications. Approximately 30% of human population are persistent carriers of *Staphylococcus aureus*and the factors that determine colonization without symptoms are largely unexplained (Mulcahy *et al.*, 2012; Mulcahy & Mcloughlin, 2016).However Variability in host adhesins, immune response, reduced expression of antimicrobial peptides in nasal secretions, polymorphisms in the genes encoding the glucocorticoid receptor, C-reactive proteins, interleukin-4 and complement inhibitor proteins have been associated with persistent nasal carriage (González-Zorn *et al.*, 2005; Akker *et al.*, 2006; Emonts *et al.*, 2008; Ruimy *et al.*, 2010).

In Health Care Workers and clinical students the carriage rate ranges from 17%-40% (Abu-ali et al., 2017; Chen, Chen, & Huang, 2012; Mcanally et al., 1984). Colonization of Health Care Workers with *Staphylococcus aureus* is a prerequisite for subsequent endogenous infection and dissemination of the strains to hospital environment (Wertheim, Melles, Vos, Leeuwen, *et al.*, 2005).

Staphylococcus aureus first developed resistance to penicillins by production of penicillinases and discovery of penicillinase resistant penicillins (methicillins) provided a temporary respite. However by 1961 slightly after introduction of methicillin, cases of

methicillin resistant were described in England(Lowy, 1998).Infections by Methicillin resistant strains (MRSA) were confined to healthcare environments and were therefore considered to be healthcare-associated infections (HAIs) (Lowy, 1998). Risk factors for MRSA infections included elderly, prolonged hospitalization, ventilatory support, indwelling catheters, and long-term residence in health care facilities (Lowy, 1998). However by 1990, MRSA epidemic infections emerged among populations lacking the risk known factors (Herold *et al.*, 1998; Popovich, Weinstein, & Hota, 2008 and Seybold *et al.*, 2006). Such strains were therefore named Community–Associated MRSA (CA-MRSA) and are reported to be more virulent than typical Hospital Associated MRSA (HA- MRSA) because of their production of panton-valentine Leukocidin (PVL) (Boyle-vavra & Daum, 2007; Lina *et al.*, 1999).

Classical Methicillin resistance by *S. aureus* is due to possession of mecA gene that codes for refractory penicillin binding protein 2a (PBP2a) instead of the normal penicillin binding protein 2 (PBP2) (Ito, Tsubakishita, Han, & Hiramatsu, 2013; Nester *et al.*, 2004; prescott, Harley, & Klein, 2002; Sangappa & Thiagarajan, 2012). MecA gene is carried on a mobile genetic element called staphylococcal cassette chromosome mec (SCCmec) and besides methicillin resistance, SCC elements carry resistance to other antibiotics and heavy metals (Ito, Tsubakishita, Han, *et al.*, 2013).

To-date MRSA is currently the most commonly identified antibiotic-resistant pathogen in many parts of the world (Shibabaw *et al.*, 2013).In Uganda, the research by Ojulong *et al*, (2009) reported MRSA of prevalence of (31.6%) in post operative wound infections in Mulago hospital (Ojulong, Mwambu, Joloba, Bwanga, & Kaddu-Mulindwa, 2009). A subsequent study reported a prevalence of 46% in Mulago hospital (Kateete *et al.*, 2011). Although latter in 2013, another study in Mulago hospital revealed a reduced prevalence than the latter (37.5%) (Seni *et al.*, 2013), the differences might be attributed to differences in season and study settings. Further still , a different study in South western Uganda found prevalence of MRSA as (28%) in Mbarara (Iramiot *et al.*, 2014). Despite differences in figures, all the studies indicated high MRSA prevalence and this suggests that the transmission rate of MRSA within Ugandan health care facilities is exceedingly high. The increasing resistance of this pathogen to various antibiotics complicates

treatment of *S aureus* infections (Wertheim & Melles, 2005) and this is an increasing problem in health care facilities (Hal, Stark, Lockwood, Marriott, & Harkness, 2010). According to Kirecci *et al.*, 2010, Healthcare workers (HCWs) constitute an important reservoir of *S. aureus* and MRSA(Kirecci, Ozer, Aral, & Miraloglu, 2010).This study determined the nasal carriage rate of MRSA among healthcare workers at the Kampala International University Teaching Hospital and the antimicrobial susceptibility patterns of the isolates with the hope that infection control programs may base on the findings to formulate guidelines as well as to decolonize the colonized healthcare workers.

1.2 The Problem statement

Transmission of MRSA from colonized health care workers to other people has been reported by many studies (Hetem *et al.*, 2012; Shibabaw *et al.*, 2013). Several studies have reported high prevalence of MRSA in Uganda mainly by analysis of samples from patients' wounds and environmental samples. Methicillin Resistant *Staphylococcus aureus* is currently the most commonly identified antibiotic-resistant pathogen. Resistance to Methicillin class of drugs is due to possession of MecA gene and such strains are also resistant to other antibiotic classes. There is insufficient literature about MRSA and its genetic basis among Health Care Workers especially in Uganda, making it hard to control MRSA transmission because of this inadequacy. There is thus a need to search for the origin and reservoir of the pathogen which is helpful in epidemiological and prediction of outbreaks.

1.3 Overall objective

To determine the prevalence of nasal carriage and antibiotic susceptibility pattern of *S. aureus* including MRSA from Healthcare workers in Kampala International University Teaching Hospital

1.3.1 Specific objectives.

(i) To determine carrier status of *Staphylococcus aureus* in the nasal cavity of KIU TH health care workers.

(ii) To determine antimicrobial susceptibility patterns of *Staphylococcus aureus* isolates.

(iii)To determine the genetic basis of methicillin resistance in *Staphylococcus aureus* strains.

1.4 Research questions.

i. What is the proportion of KIU TH health care workers that harbor *S. aureus* in their nostrils?
ii. What proportion of the *S. aureus* isolates are methicillin resistant?
iii. What is the antimicrobial susceptibility profile of *Staphylococcus aureus* isolates?

1.5 Scope of the study

1.5.1 Geographical scope

This study was done at Kampala International Hospital Western Campus, located in Ishaka town, southwestern Uganda. The study involved collecting Nasal swab specimens from consenting Health Care Workers who deal directly with patients. The study was done in different department like special clinics (dental, ear-nose and throat, ophthalmology and mental health), Out Patients' Department, Pediatric ward, surgical ward, obstetrics and Gynecology, laboratory, medical ward and accident/emergency department.

1.5.2 Content scope

The study collected the demographics (sex, age and occupation) of the participants as probable factors responsible for colonization by *Staphylococcus aureus*. Nasal swab specimens were collected and cultured to isolate *Staphylococcus aureus*.The isolates were screened for Methicillin resistance using phenotypic and genotypic methods. The antimicrobial susceptibility pattern of all the *Staphylococcus aureus* isolates was determined using Kirby_Bauer disc diffusion method on Muller-Hinton agar.

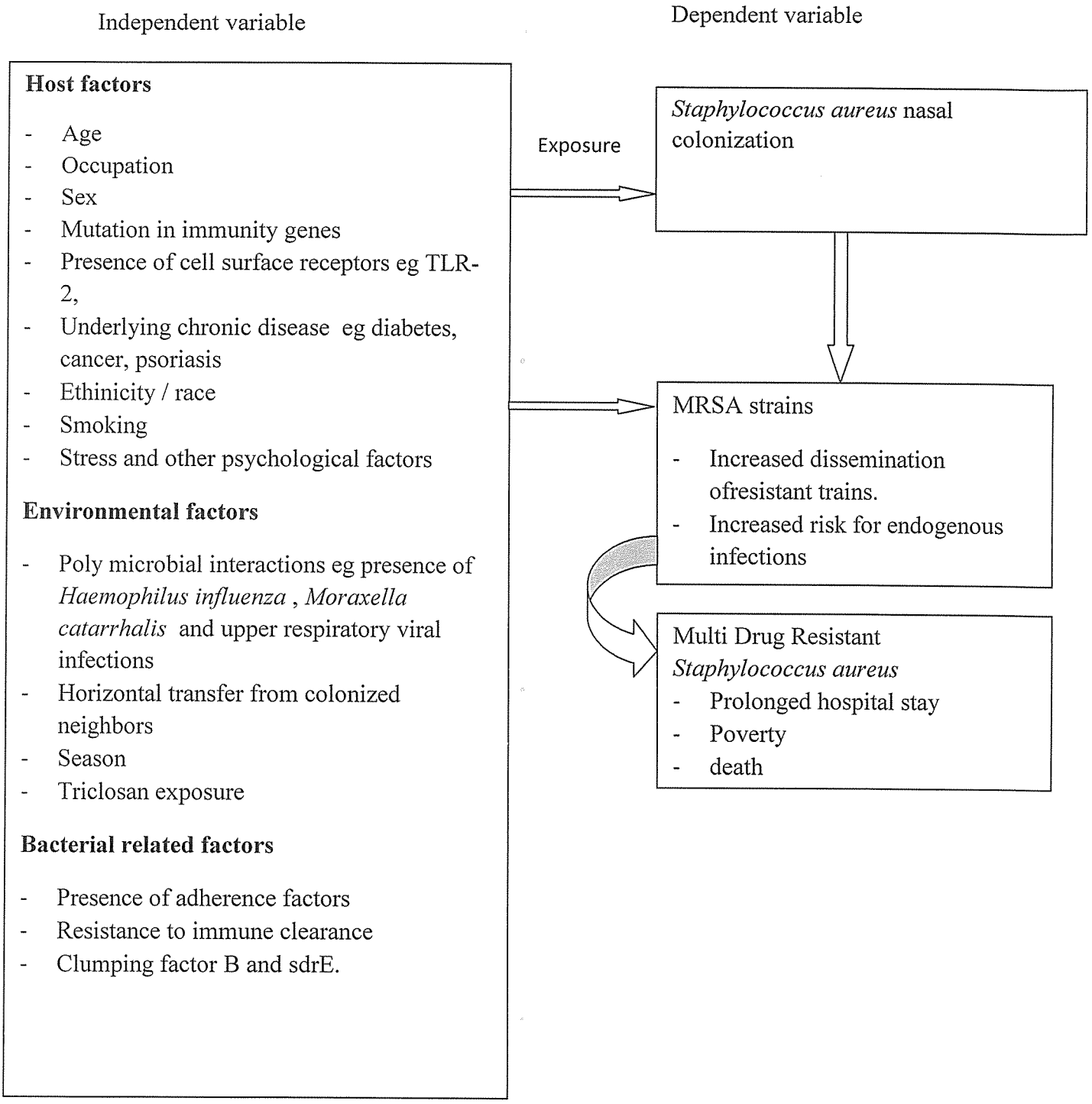
1.5.3 Time scope

The study was done in period of one year between September 2016 and July 2017

1.6 *Significance* of the study

Literature on the carrier status of *S. aureus* among health care workers is still scanty; this study is thus thought to contribute to the understanding of the epidemiology such as reservoirs of the pathogen. The knowledge of the carrier status of MRSA and its genetic profile especially possession of MecA and antimicrobial susceptibility pattern may be helpful in preventing outbreaks as carriers may be excused from sensitive places where patients are highly vulnerable. The findings may also be helpful in formulating infection control measures and effective treatment options to MRSA.

1.7 conceptual framework



Source: Done literature by the researcher based on studies by Kirecci *et al.*, 2010

Description of the conceptual frame work

Whereas *Staphylococcus aureus* is a pathogen, it colonises health people without causing disease. Some people are naturally carriers and get re-colonized after decolonization while others even resist experimental colonization(Brown, Leech, Rogers, & Mcloughlin, 2014). This suggests host range specificity for organism. Despite prevailing conditions and factors responsible for colonization largely remain unkown, biotic and abiotic factors have been suggested. For example Patel *et al.*, (2015) and Sivaraman *et al.*,(2009) found out that host factors such as age, ethnicity, presence of receptors like TLR-2, Immunity and Intravenous drug use contributed to colonization (Patel, Alvarez-fernandez, Jennings, Mccormick, & Chonmaitree, 2016; Sivaraman, Venkataraman, & Cole, 2009). Other factors such as season, exposure to triclosan, polymicrobial interactions and upper respiratory tract viral infection have been reported *(Sivaraman et al.*, 2009; Syed, 2016)The overall carriage rate of *Staphylococcus aureus* and MRSA is higher in HCWs than in the other people (Kirecci *et al.*, 2010). This probably is due to frequent exposures to the pathogens in hospital setting. Colonized Health Care Workers can be sources for endogenous infections and dissemination of the strains to hospital environment (Wertheim, Melles, Vos, Leeuwen, *et al.*, 2005). MRSA strains are a threat because of their ability to resist other antibiotic classes in addition to penicillins (Sangappa & Thiagarajan, 2012). Therefore infections with MRSA strains results into prolonged hospital stay, increased cost of treatment and even death.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of *Staphylococcus*

Staphylococcus is Gram-positive bacteria arranged in grape-like irregular clusters and grows readily on most bacteriologic media under aerobic conditions. They grow rapidly at 37 °C and metabolically, ferments carbohydrates producing pigments that vary from white to deep yellow. The pigments are best formed at room temperature (20–25 °C). *S. aureus* usually forms gray to deep golden yellow colonies which on solid media are round, smooth, raised, and glistening.

The pathogenic strains of *Staphylococcus aureus* often hemolyze red blood cells, coagulate plasma, and produce a variety of extracellular enzymes and toxins. They are the leading cause of surgical wound infections and bacteremia in hospitals. *Staphylococcus aureus* is coagulase positive and this characteristic differentiates it from two other clinically important species *ie Staphylococcus epidermidis,* and *Staphylococcus saprophyticus* (Bhutia, Singh, Biswas, & Adhikari, 2012)

2.2 Carriage of *Staphylococcus aureus* by healthy people

Staphylococcus aureus is a pathogen but also a normal flora in healthy people (Gordon & Lowy, 2008; Truong *et al.*, 2011). This organism colonizes naturally the skin and nasal mucosa of human beings(Williams, 1963) Both host and bacterial factors determine the carriage although host's phenotypic determinants are largely unknown, there seems to be genetic factors predisposing individuals to colonization. This is supported by Studies by Brown and colleagues who demonstrated that after decolonization, persistent carriers often become re-colonized with their prior *S. aureus* strain, whereas non-carriers resist experimental colonization (Brown, Leech, Rogers, & Mcloughlin, 2014).Some of the host factors known to predispose individuals to *S. aureus* colonization include variability in host adhesins, immune response or secretion of antimicrobial molecules (Williams, 1963). Persistent carriers often carry a single strain whereas intermittent carriers can be

colonized with unrelated strains over time, suggesting co-evolution of bacterial and host factors (Peacock *et al.*, 2003).

The association between *S.aureus* nasal carriage and Staphylocaccal disease was first reported in 1931 by Danbolt who studied furunculosis as cited by (Wertheim & Melles, 2005). Studies show *S. aureus* nasal carriage rates among health populations ranges between 20% and 55% (Rasamiravaka *et al.*, 2013), with about 20% (range12% to30%) of individuals being persistent *S. aureus* nasal carriers and approximately 30% being intermittent carriers (range 16% to 70%) (Kluytmans & Verbrugh, 1997). Although both Methicillin-Susceptible *S. aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* colonises people, (MRSA) can colonize healthy people at a lower rate, about 1% to 8%, (Wertheim & Melles, 2005). Healthcare workers (HCWs) constitute an important reservoir of *S. aureus* (Kirecci *et al.*, 2010). Several studies have reported that the rate of the nasal carriage of *S. aureus* among the HCWs and clinical students ranges from 17%-40% *(Adesida et al.*, 2007; Chen *et al.*, 2012; Wawer *et al.*, 1998) and this is a potential risk factor for subsequent *S. aureus* infections

2.3 Resistance to penicillins by *Staphylococcus*

Penicillins are members of a larger group of antibiotics whose structure have a β-lactam ring (Nester *et al.*, 2004). Other antibiotics which have a β-lactam ring include cepharosporins, carbapenems and monobactams (Nester *et al.*, 2004). These drugs all work by inhibiting enzymes called penicillin binding proteins (PBPs) involved in formation of peptide bridges between adjacent peptidoglycan strands of the bacterial cell wall (Nester *et al.*, 2004). In 1944, most staphylococci were susceptible to penicillin G, though a few resistant strains had been observed (Jawetz, Melnick, & Adelberg, 2007). Due to over use of penicillin, 65–85% of staphylococci isolated from hospitals in 1948 were β-lactamase producers and thus resistant to penicillin G (Jawetz *et al.*, 2007).

β-lactamase degrades the β-lactam ring of the antibiotic. Its production is controlled by aplasmid and the gene that codes for beta lactamase is called blaZ and it is regulated in an operon manner by a regularory gene called BlaR1 (Sangappa & Thiagarajan, 2012). The advent of β -lactamase-resistant penicillins (eg, nafcillin, oxacilin, cefoxitin and

methicilin) provided a temporary respite (Jawetz *et al.*, 2007; Livermore, 2000; prescott *et al.*, 2002)

First isolated in England in 1961, MRSA infections were confined to healthcare environments and patients frequenting these facilities thus referred to as Healthcare-Associated Infections (HAIs) (Lowy, 1998).By 1990, methicillin -resistant strains were also isolated in the community and were called Community Associated Methicillin Resistant *Staphylococcus aureus*(CA-MRSA) (Lowy, 1998). Upto now, MRSA is known to cause of both health care and community associated infections (Bratu *et al.*, 2005; De Sousa & De Lencastre, 2003; Seybold *et al.*, 2006). Although literature is still scarce, different MRSA prevalences have been reported in different African countries, for instance, 12.7% in Ethiopia (Shibabaw *et al.*, 2013); 35.8% in Botswana (Truong *et al.*, 2011) and 46% in Uganda (Kateete *et al.*, 2011).Colonized health care workers have been implicated as major reservoirs of MRSA by different studies as reported by (Kirecci *et al.*, 2010; Shibabaw *et al.*, 2013).

Beta lactam drugs including methicillin, work by binding to Peniccilin Binding Proteins (PBP2) which are membrane bound peptidases and the biochemical activity is mechanistically similar to that of serine proteases (Waxman & Strominger, 1983). These enzymes are involved in the synthesis of peptidoglycan; a cell wall material in bacteria. Resistance to methicillin by *S. aureus* is due to possession of mecA gene that codes for refractory penicillin binding protein 2a (PBP2a) which have a low affinity for β-lactam and cell wall is synthesized even in presence of the antibiotic (Jawetz *et al.*, 2007; Nester *et al.*, 2004). MecA gene is carried on a mobile genetic element known as "staphylococcal cassette chromosome *mec*" (SCC*mec*) (Chongtrakool *et al.*, 2006). According to Ito *et al.*, 2013, MecA gene is 2.1kb in length and it is expressed in an operon model and its expression is induced by the presence of antibiotics; that are detected by membrane anchored sensor kinases and transcriptional regulator called mecR1 becomes activated. When the antibiotics are absent the genes are switched off (Clarke & Dyke, 2001; Jordan, Hutchings, & Mascher, 2008; Ito, Tsubakishita, Kuwahara-Arai, Han, & Hiramatsu, 2013)

2.4Antimicrobial susceptibility testing

According to (Lalitha, 2004), antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include: (i) Diffusion (Stokes method or Kirby-Bauer method) (ii) Dilution (Minimum Inhibitory Concentration, Broth dilution,Agar Dilution) and (iii) Diffusion and Dilution (E-Test method). The results of *in-vitro*antibiotic susceptibility testing guide clinicians in the appropriate selection of drugs used for treating individual patients in specific situations. The selection of an antibiotic to use for susceptibility testing is based on the commonly observed susceptibility patterns and is revised periodically (Lalitha, 2004).

2.5 Detection of MRSA

MRSA identification is based upon phenotypic and genotypic methods

2.5.1 Phenotypic methods of detecting MRSA

S. aureus antimicrobial susceptibility testing is carried out using Kirby-Bauer disc diffusion method on Mueller-Hinton agar supplemented with 4% NaCl (Brown *et al.*, 2005). Oxacillin and cefoxitin are used in Kirby- Bauer disc diffusion method instead of methicillin because they maintain their activity during storage better than methicillin and hence more likely to detect heteroresistant strains (Mathews, Thomas, Appalaraju, & Jayalakshmi, 2010). Cefoxitin is a better inducer of *mecA* gene and gives clearer endpoints, easier to read than test with oxacillin(Anand, Agrawal, Kumar, & Kapila, 2009; Bosgelmez-Tinaz, Ulusoy, Aridogan, & Coskun-Ari, 2006; Mathews *et al.*, 2010). Tests using cefoxitin are more sensitive, reproducible and give accurate results than tests with oxacillin (Bhutia *et al.*, 2012). It has therefore been recommended that cefoxitin can be used as a surrogate marker for the detection of methicillin resistance in the settings where PCR is not available (Anand *et al.*, 2009; Bosgelmez-Tinaz *et al.*, 2006; Cockerill, Clinical, & Laboratory Standards, 2012; Mathews *et al.*, 2010).Clinical and laboratory standards institute (CLSI) recommends that isolates being tested against oxacillin or cefoxitin should be incubated at 33-35° C (maximum of 35°C) for a full 24 hours before reading because Cells expressing hetero-resistance grow more slowly than the oxacillin-

susceptible population and may be missed at temperatures above 35°C (Cockerill *et al.*, 2012).

2.5.2 Genotypic methods of detecting MRSA

There are currently various molecular techniques available for rapid identification and characterization of MRSA strains. These include, identification based on amplification of mecA gene that codes for penicillin binding proteins (PBP2a) confering resistance to methicillin, SCCmec analysis, spa typing, and detection of PVL (Chongtrakool *et al.*, 2006; McClure *et al.*, 2006).

2.5.2.1 SCCmec analysis

SCCmec is a mobile genetic element that carries mecA gene. There exists many structurally-distinct SCC*mec* elements in staphylococcal species but they all carry two essential components; *mec* gene complex that encodes methicillin resistance determinant and *ccr* gene complex that encodes *ccr*(s) (Ito, Tsubakishita, Kuwahara-Arai, *et al.*, 2013). Besides methicillin resistance, SCC elements carry resistance to other antibiotics and heavy metals (Sangappa & Thiagarajan, 2012). Multiple types of SCC elements seem to have evolved through repeated horizontal genetic transfer among various staphylococcal species. Six types of SCC*mec* have been recognized (Oliveira, Milheirio, & de Lencastre, 2006). The two smallest SCC*mec* types, SCC*mec* IV and SCC*mec* V, have been associated with CA-MRSA (Boyle-vavra & Daum, 2007; Vandenesch *et al.*, 2003). HA-MRSA strains carry a relatively large staphylococcal chromosomal cassette *mec* (SCC*mec*) belonging to type I, II, or III. While CA-MRSA carry smaller SCCmec types (Sangappa & Thiagarajan, 2012). Therefore by analysis of SCCmec, CA-MRSA differentiated from HA-MRSA.

2.5.2.2 Detection of PVL

Panton-Valentine leukocidin (PVL) is a two-component, pore-forming, cytolytic toxin that targets mononuclear and polymorphonuclear cells and causes cell death by necrosis or apoptosis (Boyle-vavra & Daum, 2007). The toxin consists of two synergistic proteins, LukS-PV and LukF-PV, encoded by the *pvl* genes *lukF* and *lukS*, which are carried on a

temperate bacteriophage (Said-Salim *et al.*, 2005; Vandenesch *et al.*, 2003). The expression of PVL has been strongly associated with CA-MRSA (Boyle-vavra & Daum, 2007).

2.5.2.3 Spa typing

Staphylococcal protein A is covalently anchored to the peptidoglycan. 90% of it is found in the cell wall and the remaining 10% is found free in the cytoplasm of the bacteria. In some strains especially MRSA protein A is unable to adhere to the cell wall and is released into the media (secretory protein A) (Movitz, Masuda, & Sjöquist, 1979). Spa is an important virulence factor which enables *S. aureus* to evade host immune responses by disrupting opsonization (Nester *et al.*, 2004). Isolates of *S. aureus* characterized by *spa* typing and Genotypes are known as "spa-types". Spa typing involves DNA sequencing of short sequence repeats in the polymorphic X region of the protein A gene (*spa*) of *S. aureus* (Votintseva *et al.*, 2014) and the sequence and order of specific repeats determines the *spa* type However weakness of current spa-typing primers is that rearrangements in the IgG-binding region of the gene cause 1-2% of strains to be designated as "non-typeable" (Votintseva *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

*3.*1 Study Design

This was a cross sectional study involving the collection of Nasal swab specimens from health care workers, who directly deal with patients. The participants included Nurses, paramedical officers laboratory technicians and doctors. Oral and written informed consent was sought and obtained from the participants prior to sample collection. The nasal swab specimens were collected following previously described procedure (Shibabaw *et al.*, 2013). Isolation of *Staphylococcus aureus* from the samples was done following described bacteriological methods. Antimicrobial susceptibility patterns of the *Staphylococcus aureus* isolates were done using Kirby- Bauer disc diffusion method. Screening for Methicillin Resistant *Staphylococcus aureus* was done by amplification of mecA gene using PCR.

*3.*2 Study area

The samples were collected from Healthcare Workers (HCWs) at Kampala International University Teaching Hospital located in Ishaka town along Mbarara – Kasese road in Bushenyi district, Southwestern Uganda. Kampala International University Teaching Hospital is large and sectioned into different departments which including Medical, general surgery, obstetrics and gynecology, pediatrics, orthopedics, Psychiatry, dentistry and Ear Nose and Throat (ENT). The hospital is staffed with about 249 health care workers who include nurses, clinical officers, laboratory technologists, pharmacists, medical officers and consultants.

3.3 Study population

Samples were collected from healthcare workers (Doctors, Paramedical officers, Nurses, and laboratory technologists) working in the hospital.

3.4 Inclusion criteria

Consenting Healthcare workers in the hospital were the participants

3.5 Exclusion criteria

Health Care Workers on antibacterial drugs were excluded from the study.

3.6 Sample size and sampling technique

The minimum sample size was determined by Slovin's formula stated as; $n = \frac{N}{1+N\,(e)^2}$; Where n = sample size, N = Population sizeand e = margin error (Tejada & Punzalan, 2012). In this study N= 249 and e =0.08

$n = \frac{249}{1+249\,(0.08)^2}$ = 96minimum number participants. This formula was preferred because according to (Tejada, Raymond, & Punzalan, 2012), it is the best formula when the study involves determination of proportion at confidence level 95%, and optimal when the proportion is suspected to be close to 0.5.

3.7 Sample collection

Informed consent was sought from the participants after which their biodata was taken. Nasal specimens were collected from the participants using a sterile cotton tip swab. This was done by rotating sterile cotton swabs in both nares of the participants. The specimens were then transported in Stuart transport medium to the laboratory for culture without any delay.

3.8 Isolation and Identification of *S. aureus*.

The specimens were inoculated on 5% blood agar and then incubated at 37°C for 18-24 hours. The colonies showing Beta hemolysis were identified further. The identification included: Gram staining, catalase test, manitol fermentation and tube coagulase (Iramiot *et al.*, 2014; Kateete *et al.*, 2010). The isolates that showed positive results for all the tests were confirmed using slidex staph plus (biomerieux, France) identified as *S. aureus*. The *S. aureus* were sub-cultured on Brain Heart infusion Broth (BHI) waiting to be used for further studies.

3.9 Determination of antimicrobial susceptibility pattern

Antibiotic susceptibility testing was carried out using Kirby-Bauer disc diffusion method on Muller Hinton agar (MHA). *S. aureus* colonies from manitol salt agar plates were inoculated in 5 mls of 0.85% saline (Cockerill *et al.*, 2012; Jean *et al.*, 2016)and the turbidity was adjusted to match 0.5 McFarland standard (1.5×10^8cfu.ml^{-1}). The sterile cotton swabs were dipped into the innoculum and then spread evenly onto MHA. The antibiotic discs: Vancomycin30 µg (Himedia, India), Ampicillin/cloxacillin 10 µg (Himedia, India), clindamycin 2µg (bioanalyse), Amoxycillin 30 µg (oxoid) and levofloxacin 5 µg, (Himedia, India), penicillin G (10 µg), cefoxitin 30 µg, Ciprofloxacin 1 µg, ceftazidime 30 µg, amikacin 30 µg, ampicillin 10 µg, and cotrimoxazole 25 µg (Himedia, India) were applied aseptically to the MHA plates. The plates were incubated overnight at 37^{0}C after which the zones of inhibition were measured using a ruler. The interpretation was according to CLSI, (2016) (Jean *et al.*, 2016) (appendix 4).

3.10 Genotypic screening forMRSA

The isolates were subjected to mecA gene detection using polymerase chain reaction (PCR).This was done in the Molecular Biology Laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University Kampala

3.10.1 Total DNA extraction using boiling method

The DNA was extracted using boiling method as previously described (Ahmed *et al.*, 2014 and Iramiot *et al.*, 2014) with some modifications. Briefly it involved centrifuging 1ml of bacterial culture in LB medium at 6800xg for 3 minutes at room temperature. The pelleted bacteria (sediment) was then re-suspended in 100µl of molecular biology grade water and centrifuged at 15000xg for 10 min. the supernatant was discarded and the sedment resuspended in 40 µl of molecular biology grade water and boiled at 100^{o}c in a water bath for 10 minutes. This was followed by cooling on ice and centrifuging at 15000xg for 10 seconds. The supernatant was then used for PCR.

3.10.2 PCR Amplification

This was done following the protocol reported by (Elhassan, Ozbak, Hemeg, Elmekki, & Ahmed, 2015). Briefly, a total volume of 25µl which constituted of 12.5µl of master mix (containing 2x taq polymerase, dNTPs and buffer) (biolabs, New England), 0.5 µl of forward primer, 0.5 µl of the reverse primer, 7.75µl of PCR water 1.25µl $MgCl_2$and2.5µl of the template DNA was used for PCR. The 533bp segment of *MecA* gene was amplified using the primer pair; F: 5'-AAAATCGATGGTAAAGGTTGGC-3' and R: 5'-AGTTCTGCAGTACCGGATTTTGC-3' (Qiagen) as previously reported (Al-Zaidi *et al.*, 2014). The PCR conditions was in accordance with (Kateete *et al.*, 2010) with some slight modifications. Briefly, it involved: initial denaturation at 94°C for 5minutes followed by 35 cycles of denaturation at 94°Cfor 30 seconds, Primer annealing at 50°C for 1 minute, extension at 72°Cfor 1 minute followed by 7 min of final extension at 72°C.

3.10.3 Detection of PCR products

The PCR products was resolved by electrophoresis at 125V for 30 minutes through 2% agarose gel prepared with TAE buffer containing 0.5mg/ml Ethidium bromide (Havaei, Moghadam, Pourmand, & Faghri, 2010; Shakeri & Ghaemi, 2014). DNA bands on the gel were viewed under UV digital imaging system. The size of PCR mecA amplicons were estimated at 533bp by comparison of their mobilities with those of 50bp ladder standard (Qiagen)

3. 11 Quality control

To avoid false positives, gloves and surgical mask were put on to avoid contamination of the samples. During sample collection I ensured that the entire cotton swab was inserted in the nasal osteum to avoid false negatives. All samples collected were cultured immediately after collection. The PCR was optimized prior to running the samples. Reference strain *S.aureus* (ATCC25923) was used as negative control (mecA negative) following (Baguma, Kiiza, & Bazira, 2016; The Clinical and Laboratory Standards Institute, 2016).Isolates were transferred to Mbarara University of science and technology to confirm identification and antibiotic susceptibility pattern.

3.12 Data management and analysis

Data was entered using Epi-data version 4.2 and was analysed using IBM SPSS version 20. Nasal carriage rate of MRSA was calculated as the proportion of individuals positive for MRSA out of the sample population. Chi-square test was used to compare the different groups of healthcare workers. All results with $p< 0.05$ were taken as significant.

3.13 Limitations and delimitations to the study

The study did not establish physiological and molecular factors that influence colonization of *Staphylococcus aureus* among Health Care Workers. Also the sample size was small and may not give the actual prevalence in the study population. However the techniques used in identification of the isolates are very sensitive and give the picture that the section of HWCs at KIU TH is colonized by Methicilin Resistant Staphylococcus aureus, thus requiring screening of all the Staff.

3.14 Ethical considerations

The ethical approval was obtained from Institutional Review Board of Mbarara University of Science and Technology (Ref. **No. 12/09-15**, appendix 1). The permission was sought from directorate of medical services of Kampala International University Teaching Hospital (**appendix** 2). Oral and written consent was obtained from the participants before starting the study. The identity of the participants and their information was protected by assigning sample identification numbers other than using names. Laboratory procedures were conducted in accordance with standard operating procedures.

CHAPTER FOUR

RESULTS

4.1 Participants baseline characteristics

In total 97 participants who included doctors, paramedical officers, nurses and laboratory personnel were involved in the study. Of these 61 (63%) were males and 36 (37%) were females. The participants were stratified according to age and working ward/department. Results are shown in table1.

Table 1: Participants' baseline characteristics

Characteristic	Males	Females	Total (%, n=97)	p-value
Profession				0.01*
Doctor	9 (90%)	1(10%)	10 (10.3%)	
Paramedic	28 (80%)	7 (20%)	35 (36.1%)	
Nurses	14 (38.9%)	22 (61.1%)	36 (37.1%)	
Lab staff	10 (62.5%)	6 (37.5%)	16 (16.5%)	
Age group				0.343
≤ 25	19 (59.4%)	13 (40.6%)	32 (33%)	
26-30	28 (65%)	15 (35%)	43 (44.3%)	
31-35	7 (50%)	7 (50%)	14 (14.4%)	
≥ 36	7 (87.5%)	1 (12.5%)	8 (8%)	
Ward/Department				0.653
Special clinics	7 (77.8%)	2 (22.2%)	9 (9.3%)	
OPD	4 (50%)	4 (50%)	8 (8.2%)	
Paediatric	12 (67%)	6 (33%)	18 (18.6%)	
Surgical	7 (50%)	7 (50%)	14 (14.4%)	
Obs and Gyne	4 (67%)	2 (33%)	6 (6.2%)	
Laboratory	10 (62.5%)	6 (37.5%)	16 (16.5%)	
Medical ward	7 (87.5%)	1 (12.5%)	8 (8.2%)	
A and E	10 (56%)	8 (44%)	18 (18.6%)	

A and E: Accident and emergency; Obsand Gyne: obstetrics and Gynecology; OPD: out patient's department; * statistically significant

4.2 Prevalence of Staphylococcus aureus nasal carriage

Results from this study showed that the nasal carriage rate of *Staphylococcus aureus* was 28.7% (28/97). When nasal carriage rate was compared across sex, no significant differences ($p= 0.458$) were observed between males (26.2%) and females (33.3%). The results showed nasal carriage rate significantly increased with age ($p< 0.01$), being highest (75%) in individuals above 35 years (Table 2). Comparisons of nasal carriages rates across the different professions did not show any significant differences ($p=0.225$). However, laboratory staff (50%) and Doctors (30%) were slightly more colonized. Similarly, the ward/department where the samples were collected did not significantly ($p=0.433$) impact the *S. aureus* positivity; Never the less samples collected from Laboratory (50%), Special Clinics staff (44.4%) and Outpatients departments were more positive for *Staphylococcus aureus.* Summary of these results are in (Table 2) below.

Table 2: Prevalence of *Staphylococcus aureus* nasal carriage

Characteristic	Number of samples	Positives, n (%)	p-value
Sex			0.458
Male	61	16(26.2%)	
Female	36	12 (33.3%)	
Age group			<0.01*
≤ 25	32	5(15.6%)	
26-30	43	12(27.9%)	
31-35	14	5(35.7%)	
≥ 36	08	6 (75.0%)	
Profession			0.225
Doctors	10	3(30%)	
Paramedics	35	8(22.9%)	
Nurses	36	9(25.0%)	
Lab staff	16	8(50%)	
Department/ward			0.201
Special clinics	9	4(44.4%)	
OPD	8	3(37.5%)	
Pediatrics ward	18	4(22.2%)	
Surgical ward	14	5(35.6%)	
Obs and Gyne ward	6	1(16.7%)	
Laboratory	16	8(50%)	
Medical ward	8	1(12.5%)	
A and E ward	18	2(11.1%)	

- A and E: Accident and emergency; Obs and Gyne: obstetrics and Gynecology; OPD : out patients department; * statistically significant

4.3 Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA)

4.3.1 Phenotypic MRSA Screening

In order to detect MRSA, cefoxitin discs were used. Among the 28 *Staphylococcus aureus* isolates, 13 (46.4 %) were confirmed as MRSA (Table 3). When the positive samples were compared across different age groups, it was shown that age significantly (p=0.001) affected MRSA carriage among the study population. However, there was no significant differences observed (p-values>0.05) among different sexes, professions and work departments/ wards despite the different percentages. The details as shown in table 3 below.

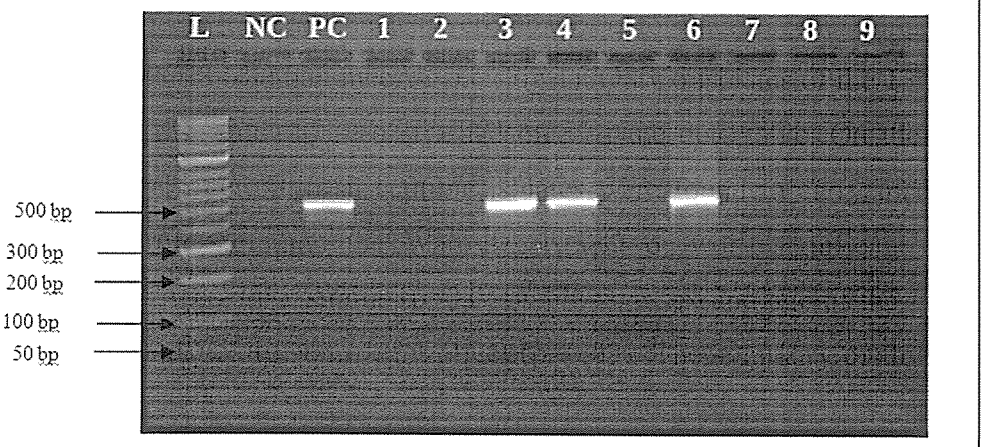
Table 3: *Staphylococcus aureus* resistance to cefoxitin (phonotypic MRSA)

Characteristic	No. of isolates	No. of MRSA	P -valve
Sex			0.404
Male	16	6 (37.5%)	
Female	12	7 (58.3)	
Age group			0.001*
≤ 25	5	1 (20%)	
26-30	12	3 (25%)	
31-35	5	5 (100%)	
≥ 36	6	4 (66.7%)	
Profession			0.448
Doctor	3	2 (66.7%)	
Paramedic	8	3 (37.5%)	
Nurse	9	5 (55.6%)	
Lab staff	8	3 (37.5%)	
Department/ ward			0.420
Special clinics	4	3 (75 %)	
OPD	3	1 (33.3 %)	
Pediatrics	4	2 (50%)	
Surgical	5	2 (40 %)	
Obs and Gyne	1	0	
Laboratory	8	3 (37.5%)	
Medical	1	0	
A & E	2	2 (100%)	

- A and E: Accident and emergency; Obs and Gyne: obstetrics and Gynecology; OPD : out patients department; * statistically significant

4.3.2 Genotypic Screening for MRSA

The *Staphylococcus aureus* isolates were analysed for MecA gene using PCR. Among the 28 isolates, 8(28.6%) had mecA gene (Fig.1). Only 6 isolates of the 13 isolates (46%) which showed resistance to cefoxitin had mecA gene detectable. On the other hand, 2 (13.3%) of the 15 cefoxitin susceptible isolates were found to carry mecA gene. The participants' profession and the Ward/department of work significantly affected the carriage of MecA positive strains ($p<0.05$) while age sex and age of the participants did not have any statistically significant effect ($p>0.05$). The summary of this analysis is in (Table 4).



L-ladder; **NC**-Negative Control; **PC**- Positive control; **3,4** and **6**- positive samples; **1,2,5,7,8**,and **9**- negative samples

Figure 1: A representative gel result demonstrating 533bp MecA gene amplicon.

Table 4: *Staphylococcus aureus* isolates with mecA gene (Genotypic MRSA)

Characteristic	Isolates with MecA (n, %)	p-value
sex		0.129
Male	03(18.8%)	
Female	05(41.7%)	
Age group		0.244
≤ 25	1 (20%)	
26-30	4 (33.3%)	
31-35	1(20%)	
≥ 36	2 (33.3%)	
Profession		0.002*
Doctors	0	
Paramedics	0	
Nurses	03(33.3%)	
Lab staff	5 (37.5%)	
Department/ward		0.033*
Special clinics	0	
OPD	1(33.3%)	
Pediatrics ward	0	
Surgical ward	1(20%)	
Obs and Gyne ward	0	
Laboratory	5 (62.5%)	
Medical ward	0	
A and E ward	1(50%)	

- **A and E**: Accident and emergency; **Obs and Gyne**: obstetrics and Gynecology; **OPD** : out patients department; * statistically significant

4.4 Antimicrobial susceptibility pattern of MRSA and MSSA isolates.

When the isolates were tested for the susceptibility to different antibiotics, MRSA isolates (mecA positive) showed higher resistance rate (Tables 5) than MSSA isolates. Since cefoxitin is also used as a predictor of MRSA, resistance rate among cefoxitin resistant and susceptible asolates was compared. cefoxitin resistant isolates showed higher resistance rate to the tested antibiotics than cefoxitin susceptible ones (Table 6). *Staphylococcus aureus* whether MRSA or MSSA isolates showed high resistance rate to Ceftazidime, Amoxicillin/ clavulanic acid, penicillin G and Cotrimoxazole. On the other hand, the isolates were very susceptible to Vancomycin, Amikacin and Levofloxacin.

Table 5: Antimicrobial Susceptibility pattern of mecA positive and negative isolates

Antibiotic	**Resistance among mecA positive, n=8**	**Resistance among mecA negative, n=20**
Clindamycin	0	0
Amoxicillin/clavulanic acid	8 (100%)	20 (100%)
Levofloxacin	1 (12.5%)	4 (20%)
Vancomycin	0	0
Amikacin	0	1 (5%)
Trimethoprom/Sulphomexazole	5 (62.5%)	6 (30%)
Penicillin G	8 (100%)	18(90%)
Ceftazidime	8 (100%)	20 (100%)
Ciprofloxacin	6 (75%)	10 (50%)

Table 6: Antimicrobial susceptibility pattern of cefoxitin resistant and cefoxitin susceptible *Staphylococcus aureus*

Antibiotic	Resistant rate (%) among cefoxitin resistant, n=13	Resistance rate (%) among cefoxitin susceptible, n=15
Clindamycin	1 (7.7%)	1 (6.7%)
Amoxicillin/clavulanic acid	13 (100%)	15(100%)
Levofloxacin	3 (23.1%)	2 (13.3%)
Vancomycin	0	0
Amikacin	1 (7.7%)	0
Trimethoprom/Sulphomexazole	10 (76.9%)	2 (13.3%)
Penicillin G	12 (92.3%)	14 (93.3%)
Ceftazidime	13 (100%)	15 (100%)
Ciprofloxacin	9 (69.2%)	7 (46.7%)

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study determined *S. aureus* nasal colonization rate among HCWs, proportion of the methicillin resistant isolates and the antimicrobial susceptibility pattern of both MSSA and MRSA isolates.

Results from this study showed that nasal carriage rate of *S. aureus* among health care workers in Kampala International Hospital; South Western Uganda was 28.8%. Different studies have reported different prevalences, for example (41.9%) in Central Uganda (18.3%) in Kenya, (28.8%) in Ethiopia, (64%) in Nigeria, (31%) in Iran and Palestine, (47.6%) in Iraq and (22%) in India (Akujobi, Egwuatu, & Ezeanya, 2013; Askarian, Zeinalzadeh, & Japoni, 2009; Kateete *etal.*, 2011; Nabil, Ali Al Laham, & Ayesh, 2017; Omuse, Kariuki, & Revathi, 2012; Shibabaw, Abebe, & Mihret, 2013;Vaidya Rutvi, Sangeeta, Sima, & Piyush, 2016). These differences probably are due to differences in the relative abundances of *S. aureus* in the respective environments of the study sites for example central versus western Uganda.

Age was associated with *S. aureus* nasal colonization rate with older people being more colonized. Related studies by Hogan *et al*, (2016) and Shibabaw *et al*, (2013) also reported the significance of age in colonization rate, although prevalence in different age groups differ from what this study report (Hogan *et al.*, 2016 & Shibabaw *et al.*, 2013). The observed high nasal carriage rate may be due to cumulative exposures to the organism which happens as one spends more time in hospital setting.

The prevalence of *Staphylococcus aureus* in this study varied according to professions ranging from 50% among Laboratory workers to 22.9% in paramedical officers. Despite these differences, there was no statistical difference. This observation agrees with what was reported by Omuse *et al.*, (2012) who observed higher prevalence of *Staphylococcus aureus* nasal carriage among Phlebotomists (Omuse *et al.*, 2012). This suggests that Health care Workers may be getting these organisms from patients as they collect laboratory samples or offer treatment. Laboratory staff are also exposed to isolates grown

in the labs especially if safety precautions are not adhered to while handling the samples/isolates thus becoming colonized by *S.aureus*. In this study the colonization rate among nurses was 25% (3rd highly colonised) as compared to study in Ethiopia, by Shibabaw *et al.*, 2013 who found 21.2% colonization rate among nurses (Shibabaw *et al.*, 2013) and were the highly colonised. The current study reports doctors to be more colonized than nurses probably due to the nature of their work for example performing operations on wounds infected by *S.aureus* and prolonged exposure to the pathogen than nurses.

Of the *Staphylococcus aureus* isolated, 46.4% showed resistance to cefoxitin (hence phenotypic MRSA) and 8 (28.6%) possessed mecA gene (hence genotypic MRSA). This may be suggestive that using cefoxitin may be more sensitive than mecA detection even though 2 of the cefoxitin sensitive isolates had mecA gene. Comparing these finding with other studies, it can be shown that there are slight differences in the prevalence for example Zorgani *et al.*, (2009) reported 36.8% in Libya, Gebreyesus *et al.*, (2013)reported 14.1% in northern Ethiopia while Shibabaw *et al.*, (2013) reported 44.1% in north east Ethiopia (Gebreyesus, Gebre-Selassie, & Mihert, 2013; Shibabaw *et al.*, 2013). All these differences in the findings may be due to different geographical distributions of *S. aureus* and relative prevalence of MRSA in different places. This claim may get support from the fact that in Kenya (Omuse *et al.*, 2012) reported 0% MRSA carriage rate among HCWs.

Age was associated with *S. aureus* nasal colonization rate among HCWs ($p<0.01$), with older ages being more colonized than the young ages. This is in agreement with (Hogan *et al.*, 2016 & Shibabaw *et al.*, 2013), who also reported the significance of age in colonization rate. The high nasal carriage rate in older ages may be due to cumulative exposures to the organism which happens as one spends more time in hospital setting.

Both the participants' profession and the ward/department where they work statistically affected the genotypic MRSA carriage rate ($p<0.05$), with only nurses and Laboratory staff carrying mecA positive strains. This study concur with other studies done (Nabil *et al.*, 2017; Shibabaw *et al.*, 2013), that established nurses to be more MRSA carriers than

other professionals. The higher prevalence of genotypic Methicillin Resistant *Staphylococcus aureus* isolated from lab staff and nurses than other professions could probably be due to frequent exposure to patients. This may also be attributed to the individuals not adhering to safety precautions; ; for instance laboratory staff may also be exposed to laboratory strains.

In the current study, five cefoxitin resistant isolates were negative for MecA. This has been observed in other studies (Olayinka, Olayinka, Obajuluwa, 2009;Broekema, Van, Monson, Marshall, & Warshauer, 2009). When Nitrocefin assay was performed on these isolates they showed hyper production of type A beta lactamases. Genome sequencing of another *Staphylococcus aureus* strain with this trait (called LGA251) found a mecA homologue which was 69% identical to mecA. This homologue is currently called mecC and also confers resistance to methicilin drugs (Bonnedahl *et al.*, 2014)."Auxiliary genes" identified by Tn551 mutagenesis have also been shown to confer resistance to Methicillin drugs in addition to mecA gene (Choon Keun Kim, Catarina Milheiriço, Hermínia de Lencastre, 2017). This shows that methicillin resistance is complex and changing as new strains are evolving different mechanisms distinct from classical mecA gene.

Despite Many studies reporting cefoxitin as surrogate marker for mecA, in the current study 2 (7%) of isolated *Staphylococcus aureus* were susceptible to cefoxitin but had mecA gene. This trend has been reported by other studies *(Bhutia et al., 2012; Cuirolo et al.*, 2011). This could be explained in terms of structural differences in the mecA regulatory genes (Katayama, Ito, & Hiramatsu, 2001) causing low expression.

The results showed that MRSA isolates are resistant to ceftazidime, Amoxycilin/ clavulanic acid, penicillin G and Cotrimoxazole but very susceptible to vancomycin, amikacin and Levofloxacin. Despite study by Ramdani-Bouguessa*et al*, (2006) in Algiers reporting Trimethoprim – sulfamethoxazole (Cotrimoxazole) as treatment option to multi-resistant MRSA (Ramdani-Bouguessa *et al.*, 2006), other studies by Baguma *et al*, (2016) and Seni *et al*, (2013) in Uganda and in many other countries by Breurec *et al*, (2010)(Baguma, Aidah, & Bazira, 2016; Breurec *et al.*, 2010; Seni *et al.*, 2013) this could be due to differences in the clone types.

5.2 Conclusions

The prevalence of nasal carriage of *Staphylococcus aureus* among Health Care Workers of Kampala International University Teaching Hospital was found to be 28.8%. Using cefoxitin disc diffusion method, 46% of the isolated *Staphylococcus aureus* were resistant MRSAwhile using PCR mecA detection 28.6% of the isolates MRSA. Methicillin resistant isolates were more resistant to antibiotics tested than methicillin susceptible (MSSA) isolates.

5.3 Recommendations

- There is need for follow up studies to identify persistent carriers and transient carriers in order to decide on decolonization measures.
- There is need to incorporate both mecA and mecC primers while studying methicillin resistance in order to avoid false negatives.
- Epidemiological studies should be carried out to establish the major risk factors for colonization of Health Workers by *Staphylococcus aureus* and MRSA so that their acquisition of the organisms is minimized and thus infection rates minimized.

5.4 Areas for future research

- Future studies should focus on whole genome sequencing when studying genetic basis for methicillin resistance so that the mutants and homologues of mecA gene that can as well code for resistance are identified. Also if resources are available, metagenomics can be done studying environmental samples, water samples, animal samples, clinical samples as well as samples from bodies of health people.

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List of appendices

Appendix1: Research approval by MUST_REC

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
RESEARCH ETHICS COMMITTEE
P.O. Box 1410, Mbarara, Uganda. **Tel: +256 4854 33795, ,**
E-mail: sec.rec@must.ac.ug **Fax: +256 4854 20782**

Our Ref: MUIRC 1/7 **Date:** March 11, 2016

Mr. Abimana B. Justus
KIU Western Campus

Re: Submitted protocol on "Nasal Carriage and Antimicrobial Susceptibility Pattern on Methicillin Resistant Staphylococcus aureas Among Health Care Workers at Kampala International University Teaching Hospital in Bushenyi, Uganda" No.12/09-15

Reference is made to the above protocol which was submitted to the Research Ethics Committee for reconsideration and full approval.

It is noted that you have addressed all the concerns earlier raised by the Committee.

I am glad to inform your study has been approved for a period of one year up to March 7 2017

The following documents have been approved with the application:

Document	Language	Version
Proposal	English	Version 2
Protocol form	English	Version 2
Data Collection tool	English	Version 2
Consent form	English	Version **March 2016**

You are required to register the study with Uganda National Council for Science and Technology, and submit progress and end of study reports to MUST REC.

You can now proceed with the rest of the research activities after getting permission from Uganda National Council for Science and Technology.

I wish you all the best.

Dr Francis Bajunirwe
CHAIR
MUST RESEARCH ETHICS COMMITTEE

Appendix 2: Informed consent used in the study

(23)

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
RESEARCH ETHICS COMMITTEE
P.O. Box 1410, Mbarara, Uganda
Tel. 256-4854-33795 Fax: 256 4854 20782
Email: irc@must.ac.ug mustirb@gmail.com
Web site : www.must.ac.ug

INFORMED CONSENT DOCUMENT

This document outlines the research study and expectations for potential participants. It should be written in layman terms and typed on MUST-IRC letterhead. The wording should be directed to the potential participant NOT to IRC. If a technical term must be used, define it the first time it is used. Also, any abbreviation should be spelled out the first time it is used.

NB: All the sections of this document must be completed without any editing or deletions

*Please **use a typing font** that is easily distinguishable from the questions of the form*

Study Title: *It should be the same as on all other documents related to the study*

NASAL CARRIAGE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG HEALTH CARE WORKERS AT KAMPALA INTERNATIONAL UNIVERSITY TEACHING HOSPITAL IN BUSHENYI, UGANDA

Principal Investigator(s): ABIMANA B. JUSTUS

INTRODUCTION

What you should know about this study:

- You are being asked to join a research study.
- This consent form explains the research study and your part in the study
- Please read it carefully and take as much time as you need
- You are a volunteer. You can choose not to take part and if you join, you may quit at any time. There will be no penalty if you decide to quit the study

Provide here a brief background to the study

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a serious clinical problem worldwide since the1980s and continues to rise globally with significant regional variations.

Leave blank for IRC office only:	IRC OFFICE USE ONLY:
MUST-IRC Stamp:	APPROVAL DATE: March 11, 2016 APPROVED CONSENT IRB VERSION NUMBER: March 2016 PI NAME: Abimana Justus IRB NO: 209-15

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are resistant to all β-lactam antibiotics. Studies have shown that MRSA are not only resistant to β-lactam but also to a variety of other antibiotic classes. Transmission of MRSA from colonized health care workers to their households has been reported. It is therefore hoped that this study will establish the occurrence and carriage rate of MRSA in KIU-TH health care workers and their antimicrobial susceptibility profiles may contribute to establishment of the effective treatment, and to strengthen infection control measures to minimize the rate of transmission of MRSA.

Purpose of the research project: *Include a statement that the study involves research, estimated number of participants, an explanation of the purpose(s) of the research procedure and the expected duration of the subject's participation.*

This project is part of requirement for my academic award and is expected to last for three month. I hope to research on five participants per day and in total I am targeting 153 participants.

Why you are being asked to participate: *Explain why you have selected the individual to participate in the study.*
You have been selected because this study is targeting health workers and as a health worker you qualify for inclusion.

Procedures: *Provide a description of the procedures to be followed and identification of any procedures that are experimental, clinical etc. If there is need for storage of biological (body) specimens, explain why, and include a statement requesting for consent to store the specimens and state the duration of storage.*

As a participant, you will be asked few questions regarding your profession and there after nasal swab specimen will be taken from you. This specimen will be carried to the laboratory to screen it for methicillin resistant *Staphylococcus aureus* (bacteria).

Leave blank for IRC office only: MUST-IRC Stamp:	IRC OFFICE USE ONLY: APPROVAL DATE: March 11, 2016 APPROVED CONSENT IRB VERSION NUMBER: March 2016 PI NAME: Abimana Justus IRB NO: 12/09-15

Risks / discomforts: *Describe any reasonably foreseeable risks or discomforts-physical, psychological, social, legal or other associated with the procedure, and include information about their likelihood and seriousness. Discuss the procedures for protecting against or minimizing any potential risks to the subject. Discuss the risks in relation to the anticipated benefits to the subjects and to society.*

There are no risks identified from participating in this study. You can withdraw from the study any time.

Benefits: *Describe any benefits to the subject or other benefits that may reasonably be expected from the research. If the subject is not likely to benefit personally from the experimental protocol note this in the statement of benefits.*

The study is beneficial at all levels. for instance if you are found colonized by MRSA, you become aware of your carrier status and transmission of the pathogen to your family and your patient is minimized. Policy makers will devise appropriate measure to contain the organism at minimum cost.

Incentives / rewards for participating: *It is assumed that there are no costs to subjects enrolled in research protocols. Any payments to be made to the subject (e.g., travel expenses, token of appreciation for time spent) must also be stated, including when the payment will be made.*

Your participation is voluntary and there is no economic gain from this study.

Protecting data confidentiality: *Provide a statement describing the extent, if any, to which confidentiality of records identifying the subjects will be maintained. If data is in form of tape recordings, photographs, movies or videotapes, researcher should describe period of time they will be retained before destruction. Showing or playing of such data must be disclosed, including instructional purposes.*

Data will be entered in a computer with pass word known only to me the Principal investigator (PI). Hard copies of the data will be kept in lockable shelves in the office accessible by only the PI. After analysis the data will be kept for maximum of 5 months

Protecting subject privacy during data collection: *Describe how this will be ensured.*

Leave blank for IRC office only:	IRC OFFICE USE ONLY:
MUST-IRC Stamp:	APPROVAL DATE: March 11, 2016
	APPROVED CONSENT IRB VERSION NUMBER: March 2016
	PI NAME: Abirwana Justus
	IRB NO: 121 09-15

Your identity will be kept incognito and any information that can reveal your identity will be strongly protected.

Right to refuse / withdraw: *Include a statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled.*

Nothing happens if you refuse to participate or withdraw from the study: you don't incur any loss.

What happens if you leave the study? *Include a statement that the subject may discontinue participation at any time without penalty or loss of benefits.*

Nothing happens if you leave the study: you don't incur any loss or cost and you can withdraw any time.

Who do I ask/call if I have questions or a problem? *Include contact for researcher or Faculty advisor and Chairman MUST-IRC*

If any question or any problem or inquiry you can contact

Mr. **ABIMANA JUSTUS (researcher)**

Kampala International University

0774373720

- **Contact for IRC office**

Dr. FRANCIS BAJUNIRWE

Chairman MUST-IRC

P.O Box 1410

Mbarara

Tel: 0485433795

Leave blank for IRC office only: MUST-IRC Stamp:	IRC OFFICE USE ONLY: APPROVAL DATE: March 11, 2016 APPROVED CONSENT IRB VERSION NUMBER: March 2016 PI NAME: Abimana Justus IRB NO: 12/09-15

What does your signature (or thumbprint/mark) on this consent form mean?

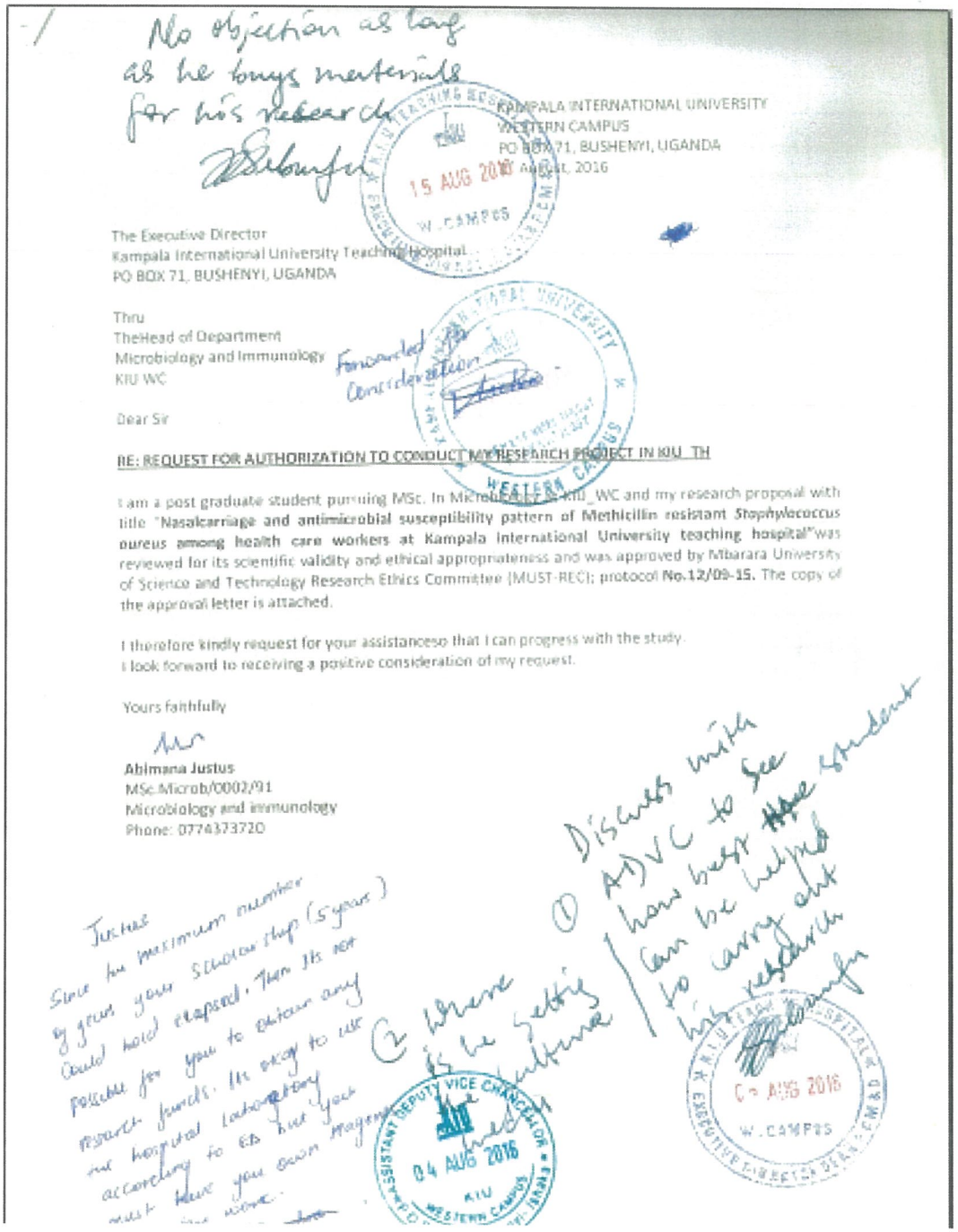
Your signature on this form means

- You have been informed about this study's purpose, procedures, possible benefits and risks
- You have been given the chance to ask questions before you sign
- You have voluntarily agreed to be in this study

Print name of adult participant	Signature of adult participant/legally Authorized representative	Date
ABIMANA JUSTUS		
Print name of person obtaining Consent	Signature	Date
Nthuma Becky		18th/09/2016
Thumbprint/mark	signature of witness	

Leave blank for IRC office only:	IRC OFFICE USE ONLY:
MUST-IRC Stamp:	APPROVAL DATE: March 11, 2016
	APPROVED CONSENT IRB VERSION NUMBER:
	PI NAME: Abimana Justus
	IRB NO: ~~12/09~~ 12/09-15

Appendix 2: Administrative clearance by KIU_TH



No objection as long as he buys materials for his research

KAMPALA INTERNATIONAL UNIVERSITY
WESTERN CAMPUS
PO BOX 71, BUSHENYI, UGANDA
August, 2016

15 AUG 2016
W. CAMPUS

The Executive Director
Kampala International University Teaching Hospital
PO BOX 71, BUSHENYI, UGANDA

Thru
TheHead of Department
Microbiology and Immunology
KIU WC

Forwarded for consideration

Dear Sir

RE: REQUEST FOR AUTHORIZATION TO CONDUCT MY RESEARCH PROJECT IN KIU_TH

I am a post graduate student pursuing MSc. In Microbiology at KIU_WC and my research proposal with title "**Nasalcarriage and antimicrobial susceptibility pattern of Methicillin resistant *Staphylococcus aureus* among health care workers at Kampala International University teaching hospital**"was reviewed for its scientific validity and ethical appropriateness and was approved by Mbarara University of Science and Technology Research Ethics Committee (MUST-REC); protocol **No.12/09-15.** The copy of the approval letter is attached.

I therefore kindly request for your assistanceso that I can progress with the study.
I look forward to receiving a positive consideration of my request.

Yours faithfully

Abimana Justus
MSc.Microb/0002/91
Microbiology and Immunology
Phone: 0774373720

Justus
Since he maximum number of your your Scholarship (5 years) could hold elapsed, then its not possible for you to obtain any research funds. It's easy to use our hospital laboratory according to its but you must have you own reagents

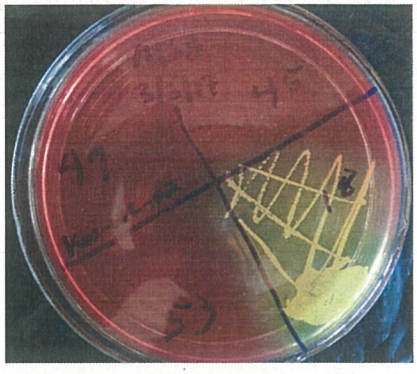
Discuss with A/DVC to see how best the student can be helped to carry out his research

Where is he getting the student

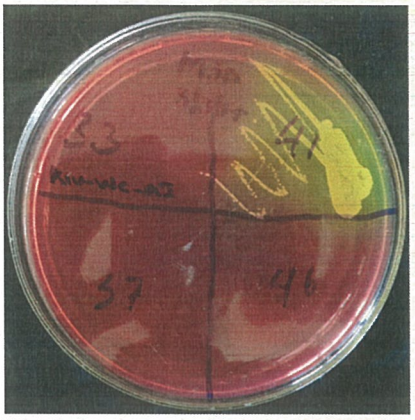
ASSISTANT DEPUTY VICE CHANCELLOR
04 AUG 2016
KIU
WESTERN CAMPUS

04 AUG 2016
W. CAMPUS

Appendix 3: Laboratory pictures during the study.

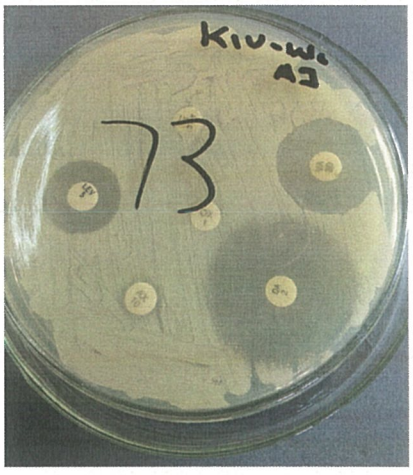


Staph *aureus* grown on manitol salt agarsample 18 is Positive the rest are negative

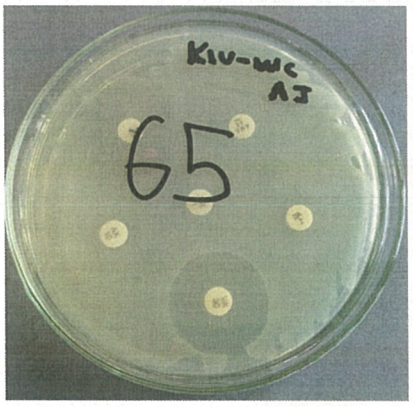


Staph *aureus* grown on manitol salt agar sample 41 is Positive the rest are negative

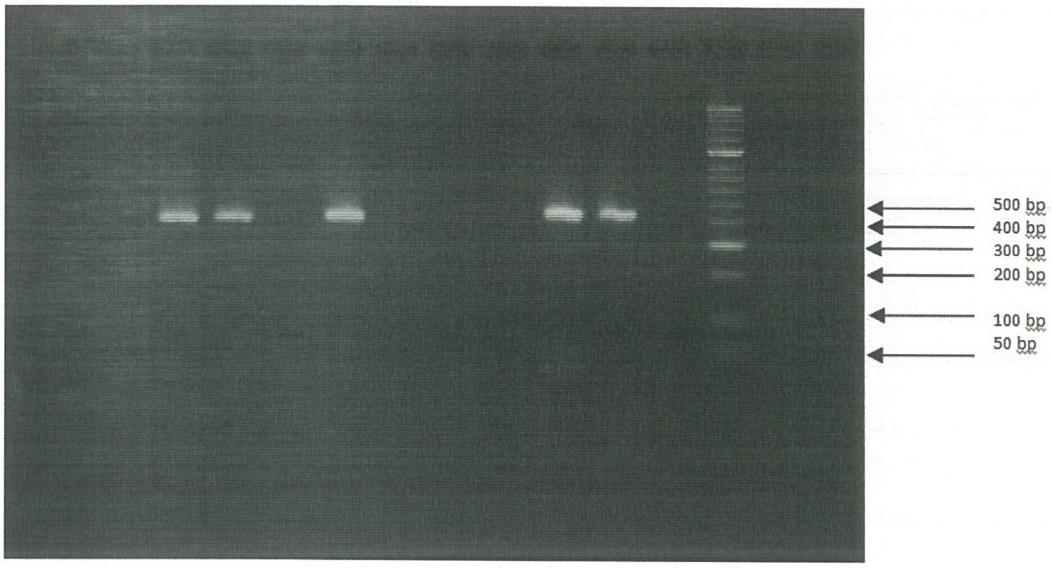
Kirby_Bauer Disc diffusion test



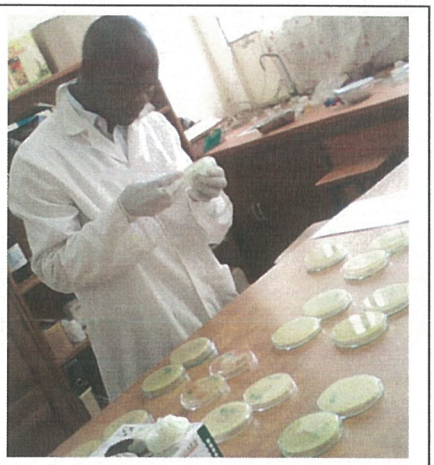
Zone of inhibition around vancomycin, clindamycin and levofloxacin



Zone of inhibition around vancomycin and small one around clindamycin.



One of the gels indicating MecA amplicon size at 533



Making observation together

Reading Kirby_Bauer Disc Diffusion Results

Appendix 4: Antimicrobial Susceptibility interpretation criteria

Antibiotic (potency)	Susceptible /diameter (mm)	Intermediate/ diameter (mm)	Resistant/ diameter (mm)
Clindamycin (2µg)	≥21	15-20	≤ 14
Amoxicillin/clavulanic acid (2µg)			
Levofloxacin (5µg)	≥19	16-18	≤ 15
Vancomycin (30µg)	≥ 7		≤ 6
Amikacin (10µg)	≥17	15-16	≤ 14
Trimethoprom/Sulphomexazole (25µg)	≥16	11-15	≤ 10
Penicillin G (10µg)	≥29		≤ 28
Ceftazidime (30µg)	≥18	15-17	≤ 14
Ciprofloxacin (5µg)	≥21	16-20	≤ 15
Cefoxitin	≥22		≤ 21
Levofloxacin (5µg)	≥19	16-18	≤ 15