CARRIAGE OF *STAPHYLOCOCCUS AUREUS* AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AMONG STUDENTS OF JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, KENYA

KHASABULI OSBORN YAMAKHAKHA

MASTER OF SCIENCE

(Medical Microbiology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2019

Carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) among students of Jomo Kenyatta University of Agriculture and Technology, Kenya

Osborn Khasabuli Yamakhakha

A Thesis submitted in partial fulfilment for the Degree of Master of Science in Medical Microbiology in the Jomo Kenyatta University of Agriculture and Technology

2019

DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University.

Signature..... Date.....

Osborn Khasabuli Yamakhakha

This thesis has been presented for examination with our full approval as supervisors:

Signature..... Date.....

Dr. Caroline Ngugi

JKUAT, Kenya

Signature..... Date.....

John Kiiru Ndemi

KEMRI, Kenya

DEDICATION

This work is dedicated to my parents and siblings.

ACKNOWLEDGEMENT

The process of earning a Master's Degree and writing a thesis is long and arduous. For that reason, I would like to thank my family for the moral and financial support during this process. Without the unending support, encouragement and understanding, it would not have been possible to sail through this educational program.

I would certainly be remiss in failing to mention and express my sincere gratitude to my supportive Dr. John Kiiru and Dr. Caroline Ngugi. They have been more than supportive through their encouragement and guidance in making sure I remain on track while executing this study. Their immense knowledge in this field of study served as an aspiration to me during the entire period.

I thank all my fellow students, students attached to the lab, the JKUAT administration and the CMR staff for their support during the study period.

Last but not least, I would like to appreciate the support I received for my PCR analysis from the Environmental & Social Ecology of Human Infectious Diseases Initiative (ESEI), Grant Reference: G1100783/1. This opportunity allowed me to run all the PCR analysis without any financial constraints.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AMC	Amoxicillin-clavulanic acid
AMP	Ampicillin
AP-PCR	Arbitrarily primed polymerase chain reaction
AST	Antimicrobial susceptibility test
C	Chloramphenicol
CA-MRSA	Community Acquired Methicillin-resistant *S.aureus*
CDC	Centre for Disease Control and Prevention
CIP	Ciprofloxacin
CL	Confidence Limit
ClfB	Clumping factor B
CLSI	Clinical & Laboratory Standards Institute
CMR	Centre for Microbiology Research
CN	Gentamicin
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease

E	Erythromycin
EPEI	Ethoxylate polyethylenimine
F	Nitrofurantoin
FOX	Cefoxitin
HAI	Hospital acquired infection
HA-MRSA	Hospital Acquired Methicillin-resistant *S. aureus*
HCW	Health Care Workers
ICU	Intensive care unit
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
LZD	Linezolid
MDR	Multi-Drug Resistant
MLST	Multilocus sequence typing
M-PCR	Multiplex PCR
MRSA	Methicillin-resistant *S. aureus*
MSA	Mannitol salt agar

NaCl	Sodium chloride
NOR	Norfloxacin
OR	Odds ratio
ORF	Open reading frame
PBP	Penicillin-binding protein
PC	Personal computer
PCR	Polymerase chain reaction
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PVL	Panton-Valentine Leukocidin
REP-PCR	Repetitive element -polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNase	Ribonuclease
SCC	Staphylococcal cassette chromosome
SERU	Scientific and Ethics Review Unit
SSTI	Skin and soft tissue infections
ST	Sequence type

SXT	Trimethoprim-sulfamethoxazole
TE	Tris Ethylenediaminetetraacetic acid
TSB	Tryptone Soy Broth
UK	United Kingdom
USA	United States of America
UTIs	Urinary tract infections
UV	Ultra Violet
VISA	Vancomycin-intermediate *S. aureus*
VRE	Vancomycin-resistant enterococci
VRSA	Vancomycin-resistant *S. aureus*

ABSTRACT

S. aureus, particularly methicillin-resistant *S. aureus* (MRSA) remains an important pathogen, but little is known about its circulation among student populations in Kenya. This study was conducted to determine the prevalence, antimicrobial susceptibility profiles, the carriage and diversity of *mecA* cassettes and *lukFS-PV* genes among the *S. aureus* isolates and factors for colonization by *S. aureus* and MRSA. To achieve these, *S. aureus* isolates were recovered from nasal, phone and pen swabs. Antimicrobial susceptibility tests were done on all *S. aureus* isolates. All the *S. aureus* isolates were screened for presence of *mecA* gene, SCC*mec* elements, while a sample of the isolates was screened for presence *lukFS-PV* genes on their gene cassettes using conventional PCR methods. A sample consisting of 44 isolates of the *S. aureus* were also analyzed for genetic relatedness via conventional PCR methods. A total of 231 *S. aureus* isolates were recovered from 89 (37.6%) participants out of 237 participants. All *S. aureus* isolates were susceptible to nitrofurantoin and linezolid and 194 (84%) isolates were resistant to ampicillin. Resistances to amoxicillin, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacin and trimethoprim-sulfamethoxazole were below 20%. The MRSA prevalence was 11.3% (26/231). The *mecA* gene was present in 17 (65.4%) of the MRSA strains. The SCC*mecV* was the most prevalent, 16 (61.5%), among the MRSA. Carriage of *lukFS-PV* gene was 31.5% and 26.9% among the MRSA and MSSA strains respectively. The factors for MRSA colonization, the factors were staying in Halls E, C and B (p=0.03) and failure to disinfect phones and pens (p=0.02). Therefore, the study confirmed the circulation of MRSA among a health study student population, thus necessitating a continued surveillance to monitor circulation of MRSA among healthy students.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Methicillin-resistant *S. aureus* (MRSA) is a bacterium that has developed resistance to methicillin and other β-lactam antibiotics. The bacteria inhabit a wide range of environments ranging from environmental surfaces, human body, animals, and surfaces of various objects (David & Daum, 2010). It is now known that emergence of resistance to β-lactam antibiotics among *S. aureus* strains occurred in several waves (Chambers & Deleo, 2010). Development of resistance to methicillin occurred in the 1960s, and this marked the beginning of the second wave of antibiotic resistance after the first wave, which was marked with development of penicillin resistance. Resistance to methicillin by *S. aureus* is now classified as a serious phenomenon because majority of such strains are also resistant to other classes of β-lactam antibiotics such as oxacillin, penicillin, amoxicillin, cephalosporins and carbapenems (Chambers & Deleo, 2009). Methicillin resistance is attributed to an extra penicillin-binding protein (PBP2a), which is coded by the *mecA* gene, and has been shown to have a significant reduced affinity for many β-lactam antibiotics (Arêde *et al.,* 2012).

MRSA have been shown to cause infections both in hospitals and in the community. Traditionally, MRSA was known to be associated with healthcare facilities. This was after the strain was first isolated in the UK in 1960s (Jevons, 1961). MRSA causing infections in settings outside hospitals have been termed as community acquired MRSA (CA-MRSA) while those acquired in hospitals are referred to as hospital acquired MRSA (HA-MRSA). Typically, infections occurring in the general community setting present as skin infections, which include boils, pimples, or lesions. Often, these infections occur in areas covered by hair or at the site of

prior skin trauma. These infections are accompanied with discomfort, pain, swelling, redness, and may have pus. Infections associated with MRSA aetiology tend to begin as small skin infections, but can spread quickly, and if not treated, the infection can progress to a serious condition that may affect other parts of the body (David & Daum, 2010). As revealed by various studies, factors associated with MRSA infections include; history of skin and soft tissue infections (SSTIs) within the past one year, residence in a long-term facility of care within the past one year, recent exposure to cephalosporin, history of carriage of MRSA within the last year and a history of close contact with patients suffering from chronic illnesses (Qiao *et al.*, 2014).

Methicillin-resistant *S. aureus* infections have become a global public health concern, especially due to infections acquired in non-hospital settings (Qiao *et al.*, 2014). In Kenya, the emergence of MRSA strains accounts for the major skin and soft-tissue infections (Aiken *et al.*, 2014). Studies show that the rapidity of the extent of the regional and global spread of MRSA strains is remarkable, but few studies have been done to isolate MRSA strains in communities in Kenya. These studies have focused on secondary schools, and they have reported the circulation of MRSA in boarding high schools (Maina *et al.*, 2013; Mbogori *et al.*, 2013).

Nevertheless, inasmuch as MRSA became eminent in the previous decade, its extent in Kenya remains uncertain. Reports from studies conducted in other countries indicate high prevalence of MRSA especially among persons living in close proximity especially in educational institutions, military barracks, prisons, and health care centres (Kejela & Bacha, 2013). In this regard students residing in institutional residence halls represent a very important group because they live in close proximities in the hostel rooms. Additionally students share different objects including clothes, personal effects, phones and pens, which may be significant in facilitating spread of MRSA. Fomites such as pens and phones play a significant role in the transmission of MRSA

among students. Various studies have documented the role of pens and cellular phones in transmission of various pathogenic microorganisms in hospital settings (Arulmozhi *et al.,* 2014; Walia *et al.,* 2014). This is because pens and phones harbour microorganisms, some of which are pathogenic. The rate at which pens and cellular phones make contact with skin is high, particularly among students (Walia *et al.,* 2014). Ultimately, this facilitates person-to-person transmission of microorganisms harboured within the surfaces of cellular phones and pens. The presence of a number of such risk factors that predispose individuals to MRSA infections, and this represents a global public health concern (Watkins *et al.,* 2012). This study was aimed at determining the prevalence, antimicrobial susceptibility profiles, genetic diversity of *S. aureus* and MRSA and the demographic characteristics associated colonization of *S. aureus* and MRSA on nostrils, phones and pens of students residing in student hostels at the Jomo Kenyatta University of Agriculture and Technology.

1.2 Statement of problems

The frequency of incidence of infections caused by MRSA strains continues to grow in school settings worldwide. Schoolmates represent the risky groups for MRSA infections. It is known that students stay in close proximities, and share material such as pens and phones, which are potential fomites. Additionally, students suffering from skin and other soft tissue infections may experience a lot of discomfort, which may interfere with learning processes. Majority of the previous studies have focused on MRSA colonization among primary school children (Kejela & Bacha, 2013). Although a few studies have focused on college students or students participating in particular sporting activities, such as, football and athletics, (Jiménez-Truque *et al.,* 2017), no study has been done in Kenya to determine the prevalence of MRSA among University students. As such, the prevalence of MRSA among university students in Kenya remains unknown.

Prevalence reports are crucial towards implementation of strategies that can help in preventing the spread of MRSA among high risk groups, and perhaps prevent outbreaks of MRSA infections.

1.3 Justification of study

University students are at a high risk of MRSA colonization. This may be due to the fact that student's stay in close proximity, sometimes in crowded conditions, and tend to share personal items including the phones and pens. Infections caused by MRSA have been attributed to several effects including patient stigmatization. As such, university students suffering from MRSA-associated infections may become prone to such effects as patient stigmatization, and this may consequently hinder learning activities in significant ways, for instance, students may experience reduced concentration while studying or missed days in class due to illness. Furthermore, due to unavailability of information on the prevalence of MRSA colonization among university students in Kenya, management of MRSA-associated infections among students may be ineffective. Therefore, analysis of MRSA colonization through collection of swabs from shared items like phones and pens in addition to nasal swabs will help in recovering MRSA that may be circulating among student populations, and this will aid in determining the prevalence of MRSA among this important group. Subsequently, information on prevalence will aid in implementation of strategies that can be used to minimize and prevent further spread of MRSA among university students.

1.4 Research questions

i. What is the current prevalence of *S. aureus* and methicillin-resistant *S. aureus* circulating among the JKUAT student population?

ii. What are the present antimicrobial susceptibility profiles of *S. aureus* strains isolated from nostrils, phones and pens used by the JKUAT students?

iii. What is the carriage of *lukFS-PV* and diversity of *mecA* cassettes among the *S. aureus* isolates obtained from the JKUAT student population?

iv. What is the genetic relationship of the *S. aureus* isolates recovered from nostrils, phones and pens used by JKUAT students?

v. What are the factors associated with MRSA colonization of nostrils and contamination of phones and pens used by JKUAT students?

1.5 General objective

To determine the prevalence, antimicrobial susceptibility profiles, genetic diversity of *S. aureus* and MRSA and the factors associated with colonization of *S. aureus* and MRSA on nostrils, phones and pens of students residing in student hostels at the Jomo Kenyatta University of Agriculture and Technology.

1.6 Specific objectives

i. To determine current the prevalence of *S. aureus* in the JKUAT student population

ii. To determine the present antimicrobial susceptibility profiles of *S. aureus* strains isolated from nostrils, phones and pens used by the JKUAT students

iii. To determine the carriage of *lukFS-PV* and diversity of *mecA* cassettes among the *S. aureus* and MRSA isolates obtained from the JKUAT student population

iv. To determine the genetic relatedness of the *S. aureus* isolates recovered from nostrils, phones and pens used by JKUAT students

v. To determine the factors associated with colonization of nostrils and contamination of phones and pens used by JKUAT students

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of *S. aureus*

S. aureus is a gram-positive coccus. It can be a human commensal or a potentially deadly opportunistic pathogen. Previous studies have established that *S. aureus* is associated with causation of different community-acquired and hospital-acquired bacterial infections (Brown *et al.,* 2014). It has been linked to most cases of bacteremia, and also carries a higher mortality rate (65-70%) compared to other bacteria in the pre-antibiotic era. Recently, it has been reported that 20-40% of mortalities occurred within 30 days of infection despite appropriate management approaches (Melzer & Welch, 2013). *S. aureus* is associated with the aetiology of deep-seated infection including septic arthritis, osteomyelitis, endocarditis, pneumonia and device-related infections. It is also unusual for its propensity in causing primary bacteremia and serious infections among young people, who are otherwise healthy and those at risks. While invasive diseases are by far the most acute and severe, the greatest burdens of morbidities are attributed to skin and soft tissue infections (SSTIs) like cellulitis, furuncules, boils, impetigo and toxic shock syndrome (TSS), which are common, often chronic and frequently-occurring (Brown *et al.,* 2014). *S. aureus* may have numerous virulence factors as well as the ability to acquire determinants for antibiotic resistance (David & Daum, 2010).

2.2 Sites and patterns of colonization exhibited by *S. aureus*

Exposure of humans to *S. aureus* occurs on a frequent basis and colonization occurs either for short or long periods through the stages of human life. The primary reservoir of *S. aureus* is the anterior nares. Extra-nasal colonization occurs in sites like throat, skin, vagina, perineum and the

gastrointestinal tract (Brown *et al.,* 2014). In a study conducted by Miller *et al.* (2012), it was reported that limiting the sampling site to the nasal cavity in regards to determination of whether a person is colonized with *S. aureus* at a single point may result in missing approximately 50% of individuals colonized with *S. aureus* elsewhere. Even so, it appears that the nasal cavity site is, often, the source of inoculation for other sites through transfer by contact (Brown *et al.,* 2014). Moreover, the greater the load of *S. aureus* in the nares, the higher the probability of colonization of other body sites. The colonization may also tend to be persistent in the presence of greater bacterial loads (Miller *et al.,* 2012). Nasal carriers of *S. aureus* can be classified into two categories. These include non-persistent carriers and persistent carriers (van Belkum *et al.,* 2009). It is estimated that 20% of people are colonized persistently with a comparatively high load of *S. aureus*, and the remaining proportion is either never colonized or is colonized intermittently with low loads of *S. aureus* (Brown *et al.,* 2014).

2.3 Transmission and dynamics of colonization by *S. aureus*

Transmission of *S. aureus* tends to occur almost entirely due to skin-to-skin direct contact, or contact with fomites that have been contaminated recently (Miller & Diep, 2008). Nevertheless, even proven contact with *S. aureus* may not necessarily lead to subsequent colonization, and certain hosts persist as non-carriers. In a study conducted by Miller *et al.* (2012), it was found out that only a marginal group of patients with an initial culture-proven *S. aureus* infection on the skin remained colonized with *S. aureus* in the convalescent phase. In this same study, it was established that only 25% of patients` house hold contacts acquired the index infecting strain of *S. aureus*. In another study, the rates of colonization among pre- and post-clinical medical students were shown not to be altered by increased exposure rates to the post-clinical group to *S. aureus* in hospital settings (Chen *et al.,* 2012). Somehow, this highlights the existence of host

differences, which confer resistance to colonization. Additionally, the association between every host and their colonizing strain is highly personalized (Brown *et al.,* 2014). The period of colonization among nasal carriers of *S. aureus* has been measured from 70 days to eight years. It appears longer, especially in persistent carriers, even though the procedure used to prove that the isolate remains the same were suboptimal, compared to contemporary methods (Hamdan-Partida *et al.,* 2010).

Some studies, especially those focused on school populations have shown that colonization by *S. aureus* appears to vary with sex of individuals. In a study conducted by Kejela & Bacha (2013), 73% of females school children and 71% of male school children were found to be colonized by *S. aureus*. In the same study, the rate of isolation of MRSA from nasal cavity was higher in females (85.2%) compared to rate of isolation among males (14.8%). Statistical analyses showed that nasal carriage of *S. aureus* was insignificantly linked to the sex of individuals (p=0.909) while there was a significant association between nasal carriage of MRSA and sex of individuals (p=0.000). It was also reported in this study that the majority of isolates were recovered from students in a class with over 60 students compared to classes with a lesser number of students (21 to 40). These results are crucial in providing insights into the dynamic of transmission of *S. aureus* in schools, where students spend most part of their time in close contact with each other, thus facilitating spread of *S. aureus* from one person to another.

2.4 Colonization of nostrils by *S. aureus*

S. aureus is a major human pathogen and has been shown to colonize mucosal surfaces and the skin in approximately 30-50% of individuals. Mulcahy *et al.,* (2012) reported that *S. aureus* asymptomatically colonizes the anterior nares and is permanently present in noses of

approximately 20% of the population, and thus represents a significant risk factor for *S. aureus* associated infections. The bacterial and host factors that facilitate colonization of the nasal cavity with *S. aureus* remain to be fully elucidated (Mulcahy *et al.,* 2012). According to research, *S. aureus* adheres to the squamous epithelial cells present in the nose. Proteins expressed on the surface of *S. aureus* such as the clumping factor B (ClfB) facilitate this interaction (Brown *et al.,* 2014). Mulcahy *et al.,* (2012) showed that loricrin, which is a major component of the squamous epithelial cell envelope represents the principal ligand for ClfB. Additionally, it was reported in the study that the interaction between loricrin and ClfB is a requirement for efficient colonization of the nasal cavity with *S. aureus* (Wertheim *et al.,* 2008).

2.5 Pens and cellular phones as fomites

Mobile phones and pens, are gaining significance as important fomites for pathogen transmission (Arulmozhi *et al.,* 2014; Walia *et al.,* 2014). Like a variety of other objects such as computer keyboards, doorknobs and telephones, mobile phones, harbour different types of bacteria on their respective surfaces, as long as the conditions on the surface of conditions for survival of the bacterial cells (Selim & Abaza, 2015). Nevertheless, because these items are "mobile or portable", they tend to be stored in pockets or bags, are handled more often, and are held in close contact or sometimes in contact with the face or mouth raising the chances of self-contamination. Due to this contact, mobile phones may be important vehicles for dispersal of bacteria (Selim & Abaza, 2015). This could be attributed to the fact that these bacteria multiply at temperatures similar to the human body temperatures. Since they are kept warm in pockets, phones can be perfect breeding places for bacteria (Walia *et al.,* 2014). Pens are also stored in a similar way as mobile phones and also make contact with the skin. Katiyar *et al.* (2011) reported that pens carry microorganism causing person-to-person transmission of the same microorganisms when pens

are shared between different people. Mobile phones are also shared in a similar manner and this leads to person-to-person transmission of microorganisms harboured in the mobile phones.

In a study that was done by Walia *et al.* (2014), a significant difference was found in the distribution of MRSA ($p<0.01$) among participants using mobile phones. According to Arulmozhi *et al.* (2014), mobile phones are usually handled irrespective of the cleanliness of hands, rarely disinfected and have the potential of harbouring pathogenic bacteria. An overall rate of contamination of 94% was found for cellular phones in the study conducted by Arulmozhi *et al.* (2014). In their study, Katiyar *et al.* (2011) reported that out of six isolates of *S. aureus* isolated from pens, four of them were MRSA. It is therefore, crucial to note that student population forms a very significant study group since it may be probable that the rate of sharing of mobile phones and pens with the University setting may play a crucial role in enhancing the transmission of *S. aureus* amongst students.

2.6 Basis of resistance to β-lactam antibiotics by *S. aureus*

The genetic determinant of resistance to β-lactam antibiotics is *mecA* gene (Fuda *et al.*, 2004).The *mecA* gene lies in the SCC*mec* resistance island and is present in about 95% of isolates of *S. aureus* displaying the phenotype of methicillin resistance (Wielders *et al.*, 2002). Resistance to β-lactam has been shown not be native to *S. aureus,* but rather, it has been acquired via the *mecA* gene for more than 40 years. The *mecA* gene encodes a protein known as penicillin-binding protein (PBP), which is normally designated as PBP2a. *S. aureus* usually produces four PBPs namely, PBP1, PBP2 PBP3 and PBP4 that are anchored on the cytoplasmic membrane (Navratna *et al.*, 2010). Penicillin-binding proteins function in assembly and regulation of the stages of synthesis of the bacterial cell wall. Whereas the four PBPs are susceptible to alteration

by β-lactam antibiotics resulting in death of bacterial cell, PBB2a is refractory to the action of all presently used β-lactam antibiotics. The PBP2a has the ability of taking over the functions of the four staphylococcal PBPs during exposure to β-lactam antibiotics (Kondo *et al.,* 2007).

The *mecA* gene is known to be carried on a peculiar type of mobile genetically element inserted into the staphylococcal chromosome, known as the staphylococcal cassette chromosome *mec* (SCC*mec*) elements (Katayama *et al.,* 2000). The SCC*mec* elements share four characteristics. First, they carry the *mec* gene complex, which consists of methicillin-resistance determinant gene (*mecA*) as well as its regulatory genes and insertion sequences; second, they carry the *ccr* gene complex responsible for mobility of the element and its associated sequences; third, they have distinct directly repeated nucleotide sequences and inverted complementary sequences at each end; and fourth, they integrate into the 3′ end of an open reading frame (ORF), *orfX*. Despite the similarities identified above, structures of SCC*mec* elements are also divergent (Kondo *et al.,* 2007).

2.7 Diversity of SCC*mec* elements in Kenya

In Kenya, the few studies which sort to examine the diversities of SCC*mec* elements among isolates have revealed the existence of diverse types of these elements. In a recent study that analyzed isolates obtained from a government hospital and a private referral hospital based within the Nairobi, the SCC*mec*III [3A] was found to be more prevalent followed by SCC*mec*IV [2B]. The predominance of these two SCC*mec* elements was attributed to their small sizes, which facilitated their transmission in both community and hospital settings (Omuse *et al.,* 2016). A study done previously on isolates recovered from a government hospital setting also revealed the existence of SCC*mec*III among all the MRSA isolates, which were only six in

number (Aiken *et al.*, 2014). In a study done by Maina *et al.*, (2013), 75.4% of *S. aureus* isolates obtained from a hospital setting harboured SCC*mec*II, 7.2% harboured SCC*mec*I and 1.4% harboured SCC*mec*II in their genome. Based on this information, it is clear that previously published studies done in Kenya on the diversity of SCC*mec* elements have only focused on isolates recovered from patients and the community MRSA component remains uninvestigated. Additionally, these studies have revealed the existence of SCC*mec* types II, III and IV. As such, there is lack of information on the SCC*mec* types harboured in *S. aureus* isolates circulating in otherwise healthy persons outside hospital settings in Kenya.

2.8 Carriage of *lukFS-PV* genes among *S. aureus* isolates found in Kenya

The *lukFS-PV* genes encode for the Panton-Valentine Leukocidin (PVL), which is a pore-forming toxin with cytolytic activities on defined cells of the immune system including monocytes, macrophages and neutrophils (Yoong & Torres, 2013). As reported by Shallcross *et al.* (2013) the PVL exhibits the propensity for causing severe, and in most cases, recurrent SSIs. Previously, Vandenesch *et al.* (2003) reported that the *lukFS-PV* genes are primarily carried by the CA-MRSA. However, reports from West and Central Africa have showed that approximately 40% of MSSA recovered from hospital settings carry the *lukFS-PV* genes in their genomes. Of importance to note also is that, several studies have reported that carriage of the *lukFS-PV* genes is significantly associated with the MRSA strains linked to the causation of severe invasive staphylococcal infections (Holmes *et al.*, 2005; Melles *et al.*, 2004). On the other hand, other studies have also revealed the lack of a significant association between the carriage of *lukFS-PV* genes and MRSA and MSSA strains (Sharma-kuinkel *et al.*, 2012; Tong *et al.*, 2012). In Kenya, varied results have been reported in respect to association of MRSA strains and carriage of the *lukFS-PV* genes. As study conducted by Aiken *et al.* (2014) on clinical isolates established that

all the MRSA isolates did not harbour the *lukFS-PV* genes while 19% of the MSSA carried the *lukFS-PV* genes in their genomes. Contrary to these findings, Maina *et al.,* (2013), in their study on clinical isolates of *S. aureus* found out that 20.3% of the MRSA strains carried the *lukFS-PV* genes. A study done by Omuse *et al.* (2013) established that there was no significant difference in terms of carriage of *lukFS-PV* genes between *S. aureus* isolates recovered from healthcare workers and those from patients (p=0.098). In this study also, a statistically significant associated was established between the carriage of *lukFS-PV* genes in *S. aureus* isolates implicated in SSIs but not in those not associated with this condition (P=0.03). These studies, however, have focused on isolates recovered from clinical settings, and as such, showing that there is a gap in information on carriage of PVL genes in isolates recovered from healthy populations in Kenya.

2.9 Epidemiology of MRSA infections

Until the 1990s, MRSA infections occurring in community settings were a rare event. Since then, there has been a rapid emergence and outbreaks of Community-acquired MRSA (CA-MRSA) worldwide. The initial reports on MRSA-associated outbreaks were from Australia and USA (Ellington *et al.,* 2010; Tenover & Goering, 2009) and such outbreaks were described among the underprivileged aboriginal communities, schoolchildren, soldiers, prison inmates, athletes and homosexuals (Fred C Tenover & Goering, 2009). Ironically, as stated in studies done by Ellington *et al.* (2009) and Diederen *et al.* (2006), the underprivileged aboriginal communities are yet to be identified as reservoirs for MRSA. The risk factors for the development of MRSA infections include; close contact with infected persons, residing in crowded facilities, sharing of personal items, participating in contact sports, poor hygiene, and living in close contact with individuals from countries with high prevalence of MRSA (David & Daum, 2010). Therefore, students residing in congested hostels represent an important population for carriage of MRSA

since all these factors apply to them, hence elevating the risk of spread of MRSA. It is also crucial to note that these observations have been instrumental in elucidating the transmission of MRSA in settings outside health care facilities.

Infections caused by MRSA have escalated to epidemic proportions worldwide. In the USA, for instance, the rise of epidemics associated with MRSA infections has been attributed to the successful spread of strains associated with the pulsed-field gel electrophoresis (PFGE) profile USA 300 that belong to MLST ST8/ SCC*mec*IV clone. These strains harbour the *lukFS-PV* genes. The respective clone has now also been reported in other European countries (Goering *et al.,* 2009). However, its occurrence in Africa remains to be properly investigated.

In Kenya, (Maina *et al.,* 2013) conducted the first study on prevalence of MRSA. Isolates were obtained from patients suffering from skin and soft tissue infections (SSTIs) in both In-patient and Out-patient settings. The prevalence of MRSA was investigated with respect to the associated infection, patient category and the type of health care facility the isolate was obtained. The findings showed that staphylococcal cassette chromosome (SCC*mec)* type II and MRSA strains carrying PVL were significant pathogens causing SSTIs infections in patients visiting various hospitals in Kenya. This study also suggested that cases of MRSA were prevalent in health care facilities that are publically funded. In a study conducted by Mbogori *et al.* (2013), MRSA were confirmed to be in circulation in different types of schools including boarding boys and girls schools, mixed boarding and day schools, and day boys` and girls` schools. While *mecA* or PVL genes were found in some isolates, MRSA strains were found to harbour both genes. Monitoring of MRSA infections in hospital and community settings is crucial. MRSA infections not only replaces the Methicillin-susceptible *S. aureus* as etiologic agent for infections, but adds

to the latter`s burden of disease. The final outcome is an increase in the incidences of infections caused by *S. aureus.*

2.10 Transmission of MRSA in community and school setting

According to CDC reports, transmission of MRSA occurs more frequently via direct skin contact (Centers for Disease Control and Prevention (CDC), 2013). Fomites have also been associated with the spread of MRSA (Arulmozhi *et al.*, 2014; Walia *et al.*, 2014). Methicillin-resistant infections occur in any setting, but several factors may facilitate the spread of MRSA. Factors associated with increased spread of MRSA include crowded living conditions, skin cuts, close skin-to-skin contacts, contaminated surfaces, and lack of good hygiene (Centers for Disease Control and Prevention (CDC), 2013; Chatterjee & Otto, 2013). University settings are among the settings where spread of MRSA can be facilitated by close contact among susceptible individuals. Students suffering from skin and other soft tissue infections may experience stigmatization due to recurrent MRSA infections (Mozzillo, Ortiz, & Miller, 2010).

The highest prevalence of MRSA infections was recorded from classrooms where the number of students was more than 60 pupils. Additionally, MRSA nasal carriage was also highest among students who came from families of five to six individuals. A different study, that was almost similar to this established that MRSA carriage was more prevalent in students between ages 10 to 19 years. The prevalence of MRSA infections was higher in females compared to their male counterparts (Rijal *et al.*, 2008). Studies conducted by (Yildirim *et al.*, 2007) also revealed the same prevalence of MRSA infections among male and female students. From the information presented above, very few researchers have focused on MRSA infections among students.

Majority of studies conducted before were mainly focused on athletes while there is a scarcity of data on students living student hostels.

2.11 MRSA infections and sports

Various studies have been conducted on MRSA infections among students in different educational institutions including high schools and tertiary institutions. These studies have revealed the burden of MRSA carriage among students. In a study conducted by (Lear *et al.,* 2011), it was revealed that outbreaks of MRSA infections have been documented among athletics, football, rugby, and soccer players in schools. In athletic settings, for instance, multiple retrospective studies have assessed the risk of MRSA colonization and infection with differing study outcomes (Huijsdens *et al.,* 2006; Kejela & Bacha, 2013; Rijal *et al.,* 2008; Yildirim *et al.,* 2007). An outbreak occurred in one of the soccer teams in Netherlands where 11 members of the team were affected. The two of the affected members lived in the same room in the college hostel (Huijsdens *et al.,* 2006). In another American school football club, a retrospective analysis of an MRSA outbreak confirmed 11 players out of 107 players suffered from MRSA infections during a match season. Surprisingly, out of the 99 players that were tested, only eight players tested positive for MRSA colonization. Researchers in this study noted that only these eight players had their swabs collected after they had taken antibiotic medication and the number of colonized may have been more (Nguyen *et al.,* 2005).This study was unique in that it identified highly resistant strains among people who had been subjected to antibiotic treatment. In another study, (Kejela & Bacha, 2013), conducted a study that focused on the MRSA colonization among school going children. The results in this study indicated that high nasal carriage of MRSA was attributed to major risk factors including sex, age, number of children per classroom, and previous hospitalization.

2.12 Burden of management of MRSA infections

Large reservoirs of MRSA isolates are presently outside of health care centres (Creech *et al.,* 2005; Farley *et al.,* 2008; Hidron *et al.,* 2005).This implies that efforts to control the spread of MRSA, must be extended beyond the hospitals to the communities (Charlebois *et al.,* 2004). Available antibiotics that can be used to treat MRSA infections are relatively few and include vancomycin, linezolid, daptomycin (Logman *et al.,* 2010). Furthermore, the available antibiotic agents have significant limitations including unfavourable returns on investment that is deterring large pharmaceutical companies from engaging in discovery of new antibiotics and failures to identify new targets for antibiotic drugs (Wenzel, 2004). Due to this, the rate of development and production of novel antibiotics has been relatively slow. Strains of *S. aureus* that are resistant to some of the available antibiotics that are effective against MRSA such as vancomycin-resistant *S. aureus* (VRSA) have been reported (David & Daum, 2010). Furthermore, David & Daum, (2010) stated that this raises the theoretical concern of the probability of emergence of untreatable multi-drug resistant (MDR) strains of *S. aureus.*

With the exerted pressure of antibiotic use, particularly with the increased of use of vancomycin in the management of MRSA infections, cases of resistance to vancomycin have been reported leading to evolution of vancomycin-resistant .*S aureus* (VRSA) (Centres for Disease Control and Prevention (CDC), 2002; Finks *et al.,* 2009). According to Hiramatsu (1998) and Hiramatsu *et al.* (1997), the initial case of Vancomycin intermediate resistance demonstrated by vancomycin-resistant *S. aureus* (VRSA) was reported in 1996 in Japan. Other strains of VRSA have been identified more commonly in various other countries including the United States in Michigan and Pennsylvania and Portugal (Gardete & Tomasz, 2014). The recognition of VRSA strains represents an ominous threat. Perhaps, a more serious concern originates from the observed slow,

but constant increase in the level of vancomycin resistance among unselected strains of *S. aureus* that can develop with vancomycin therapy. As such, clinical reliance on management of MRSA infections using vancomycin may become a challenge (Rossi *et al.*, 2014).

2.13 Methods on characterization of MRSA

Presently, there are various methods of characterization of microbial strains, consortia or communities of bacteria that can be used by epidemiologists or clinicians. Broadly, these include; molecular biological, biochemical and microbiological methods. The molecular methods are comprised of a broad range of techniques, which are based on both analysis and differentiation of microbial DNA. These methods involve the recovery and testing of source materials, which is DNA, directly from the bacterial cells, without the need of culturing. Biochemical methods comprise of different sets of methodologies. The respective methodologies are all dependent on gas chromatography and mass spectrometry for separation and precise identification of a range of biochemicals or biochemical properties of specific cellular biomolecules. Lastly, microbiological methods represent the most varied and the least useful in regards to characterization of microbial consortia. The microbiological methods are dependent on traditional tools including cell counting, microscopic examination and selective growth to provide generalized characteristics of a microbial community. The tools can also be used to provide characteristics of a small subset of members of a microbial community (Spiegelman *et al.*, 2005).

Different studies focused on MRSA have used these methods to characterize MRSA. However, the molecular techniques represent the most common methods used extensively to characterize MRSA across the globe. Molecular analysis of MRSA, previously, has been done using multi-

locus sequence type (MLST), *spa*, SCC*mec*, *agr* typing (Goudarzi *et al.*, 2016) and pulsed field gel electrophoresis (PFGE) (Champion *et al.*, 2014).

2.13.1 Multi-locus sequence type (MLST)

The MLST method characterizes bacterial isolates based on the sequences of internal fragments of ~450-bp found in seven housekeeping genes. Different sequences are assigned as distinct alleles for each of the gene fragments and definition of each isolate is done based on the alleles found on each of the seven housekeeping loci; also known as the allelic profile of the sequence type (ST) (Maiden *et al.*, 1998). The main advantages of using MLST to characterize MRSA are the possibility of rapid generation of concise results, which can allow for simple comparisons between STs (Jung *et al.*, 2011).

2.13.2 Pulsed field gel electrophoresis

The PFGE technique is based on the digestion of DNA using restriction endonucleases, which can recognize few sites along the chromosome thus generating large DNA fragments (10-800kb) that cannot be separated effectively via the conventional electrophoresis method (Schwartz *et al.*, 1964). Pulsed field gel electrophoresis has been utilized for investigation of MRSA where all isolates have been found to be typeable and standard strains have been shown to be reproducible, even after extensive subculturing (Tenover *et al.*, 1994; Tenover *et al.*, 1997). The discriminatory power of PFGE equals to or is superior to phenotypic and genotypic techniques like ribotyping, Random Amplified Polymorphic DNA (RAPD), PCR- restriction fragment length polymorphisms (RFLP) and inter-IS256 PCR (Deplano *et al.*, 1997; Kumari *et al.*, 1997; Struelens *et al.*, 1996; Tenover *et al.*, 1994). Due to its superiority regarding all the characteristics attributed to the ideal typing technique, PFGE has been proposed as the gold

standard for typing MRSA isolates (Bannerman *et al.,* 1995). The PFGE technique has been used widely for the comprehension of the epidemiology of epidemic and endemic MRSA strains (Rodriguez *et al.,* 2015; Trindade *et al.,* 2003). An interpretation scheme developed in 1995 by Tenover and colleagues is used to determine the genetic relationships among MRSA strains (Tenover *et al.,* 1995). Isolates possessing similar PFGE profiles are considered as identical in this scheme are regarded as identical. On the other hand, isolates that differ through a single genetic event, which is reflected by a difference in one to three bands, are regarded as probably related. Isolates differing in four to six bands, which represent two independent genetic events, are regarded as possibly related. Lastly, isolates with more than six bands are considered as unrelated (Trindade *et al.,* 2003).

2.13.3 Techniques involving PCR

The polymerase chain reaction gave rise to different techniques with a variety of applications including characterization of bacterial isolates. The typing techniques involving PCR are categorized into four major groups. These are PCR-RFLP, PCR-ribotyping, AP-PCR/RAPD and Repetitive Palindromic Extragenic Elements PCR (Rep-PCR) (Power, 1996). The PCR-RFLP and PCR-ribotyping are no longer applied frequently in MRSA typing (Trindade et al., 2003). The arbitrarily primed PCR (AP-PCR) technique or RAPD represents a variation of classic PCR and were proposed previously for microbial genetic analysis (Welsh *et al.,* 1990; Williams *et al.,* 1990). The technique entails the random amplification of target DNA segments using a small primer of about 10 bases with an arbitrary sequence of nucleotides. During PCR, the primer results in the amplification of a single or more DNA sequences, hence generating several fragments of different sizes that function as genetic markers. The RAPD technique generates genetic identities for all MRSA isolates. The Rep-PCR employs the use of primers developed

based on sequences of repetitive elements that are dispersed throughout the prokaryote kingdom. The repetitive elements appear to be conserved within a number of genera and species of bacteria. This way, molecular profiles of respective bacterial strains are generated, and the variations between the sizes of bands represent polymorphism in the distances between the repetitive elements found in different bacterial genomes. The Rep-PCR technique has been used to characterize isolates belonging to numerous bacterial species The Rep-PCR technique is simple and can be used to a small or large number of bacterial isolates. It has also been associated with a high discriminatory power in comparison to other typing techniques. Several studies have also shown that results obtained by Rep-PCR bear a good correlation with those generated by PFGE despite the lower discriminatory power (Trindade *et al.,* 2003). In a study carried out by Van der Zee et al. comparison was made between the results generated by Rep-PCR and those generated by other genotyping techniques, which had been used to characterize bacterial strains. Analysis showed that the discriminative power of Rep-PCR equaled that of RAPD and PFGE (Van Der Zee *et al.,* 1999). Compared to RAPD, an extra step in purification of DNA is required in Rep-PCR. Therefore, in regards to the ease of use, the utility of the RAPD is somewhat superior. Nonetheless, whereas the reproducibility of the Rep-PCR was found to be outstanding, that of RAPD was low. In comparison to PFGE, the major advantage of Rep-PCR is its simplicity and rapidity of its execution (Trindade *et al.,* 2003).

The use of characterization techniques, which are faster and highly discriminative, remains imperative. These techniques may be fundamental in times of outbreaks since they can aid the characterization of MRSA in hospital laboratories. Considering the information provided in literature and data gathered previously, Rep-PCR appears to be a promising technique in characterizing MRSA (Trindade *et al.,* 2003).

2.14 Prevention, control and current treatments of MRSA

Previously published guidelines offering comprehensive recommendations for prevention of MRSA infections are available (Calfee *et al.,* 2014). The basic practices for preventing and controlling transmission and infections caused by MRSA are as follows;

2.14.1 Conduction of MRSA risk assessments

According to available literature, the assessments should be focused on two crucial factors. Firstly, the opportunity for transmission of MRSA, which tends to be affected by the number of patients carrying MRSA and produce the MRSA transmission risk, and secondly, the approximations of facility-specific MRSA burden and transmission and infection rates, which measure the ability of the current activities in a facility initiated to contain MRSA. The findings from the risk assessments should be applied in developing a hospital's surveillance, prevention and control plan and to set goals for reduction of acquisition and transmission of MRSA.

2.14.2 Implementation of an MRSA monitoring program

According to Calfee *et al.* (2014), the established monitoring programs should be guided by two goals. These include; identification of patients with current or histories of MRSA to allow for application of infection prevention strategies as guided by health centre policies and provision of a mechanism for tracking cases of MRSA with the aim of assessing the respective transmission and infection, as well as the need for response.

2.14.3 Promotion of compliance with CDC or WHO hand hygiene recommendations

Hand hygiene represents one of the fundamental strategies for the prevention of MRSA transmission in communities and healthcare facilities (Calfee *et al.,* 2014). Person-to-person

MRSA transmission is known to occur frequently through transient colonization of healthcare professionals. Researchers have attributed rates of MRSA among patients in hospitals and communities to the efforts made to enhance hand hygiene practices (Marimuthu *et al.,* 2014).

2.14.4 Utilization of contact precautions for persons colonized or infected with MRSA

Previous studies have shown that persons interacting with those colonized or infected with MRSA often get contaminated with MRSA (Morgan *et al.,* 2012). Similarly, other studies have shown that objects found in MRSA-contaminated or MRSA-infected persons' environment become contaminated (Chang *et al.,* 2010). Placing persons colonized or infected with MRSA under contact precautions may aid in reducing person-to-person spread of MRSA (Siegel *et al.,* 2007; Siegel *et al.,* 2018).

2.14.5 Provision of education about MRSA

Education of colonized or infected persons about MRSA and recommended precautions may facilitate the reduction of anxiety linked to precautions, risk of developing symptomatic MRSA infections as well as the risk of transmission of MRSA to family members and visitors and enhance adherence to the recommended precautions (Abad *et al.,* 2010). It is crucial for patient education to be offered as soon as possible where an individual has a history of MRSA (Calfee *et al.,* 2014).

2.14.6 Treatment of MRSA infections

According to Banniettis et al. (2018), vancomycin represents the firstline parenteral antibacterial drug used to treat MRSA infections. Strains of MRSA with intermediate susceptibility to vancomycin and high-level resistance occur but less frequently (Banniettis *et al.,* 2018). On the contrary, a number of observational studies have reported a frequent correlation between failures

of vancomycin therapy and in-vitro MICs at the upper end of the official susceptibility range (Sakoulas *et al.,* 2004; van Hal *et al.,* 2012). The potential reasons for vancomycin treatment failure include failure of source control, heteroresistant subpopulations, inadequate dosing, suboptimal tissue penetration and slow bactericidal activity (Deresinski, 2007; Kollef, 2007).

Daptomycin is also considered an alternative firstline antibacterial drug for MRSA (Holland *et al.,* 2014). As discussed by Hassoun et al. (2017), the MICs for vancomycin and daptomycin are correlated, and about 15% of heterogeneous vancomycin-intermediate *S. aureus* are also resistant to daptomycin. Studies have also suggested that prior vancomycin treatment failure is correlated with the heteroresistance acquisition and reduced daptomycin therapy success levels (Kullar *et al.,* 2013; Moise *et al.,* 2013). Therefore, higher daptomycin doses (8-10mg/kg) may be necessitated for complicated and persistent MRSA infections (Holubar *et al.,* 2016).

Teicoplanin is also another alternative treatment for MRSA, particularly for patients who are refractory to vancomycin (Holland *et al.,* 2014). It is approved by the European Medicine Agency for treating bacteraemia associated with a number of Gram-positive bacteria, and is considered as safe and effective as vancomycin in treating HA-MRSA (Yoon *et al.,* 2014).

2.15 Research gaps in Kenya regarding MRSA in University settings

Data on MRSA in Kenya is scanty. The earliest reports of incidence of MRSA infections within the sub-Saharan Africa came from different hospitals including the Thika Level 5 Hospital, Kenya during the early 1990s (Aiken *et al.,* 2014). MRSA were also principally isolated from patients with burn wounds attending In-patient services at the Thika Level 5 Hospital (Muthotho *et al.,* 1995). Recent studies done in Kenya (Aiken *et al.,* 2014; Maina *et al.,* 2013; Omuse *et al.,* 2016) also analyzed isolates of a clinical origin and thus, there is a gap in the prevalence of

MRSA among isolates recovered from the general population. Only a single study done in Kenya (Mbogori *et al.,* 2013), which focused on non-clinical isolates was found. However, the isolates were obtained from fomites and not healthy people. Therefore, it is evident that none of the past studies has determined the prevalence of these strains in the university settings and the factors associated with MRSA and MDR Staphylococcal colonization in remain unknown. Also, there is lack of information on the antimicrobial susceptibility profiles of *S. aureus* isolates from the general population, carriage of *lukFS-PV* genes and genetic diversity, particularly with respect to carriage of SCC*mec* elements among these isolates. Therefore, this study, which is focused on one of the high-risk groups (students sharing hostel rooms) for MRSA colonization, aims at providing the missing information. Specifically, this study was designed to provide insights into the carriage of *S. aureus* and related MRSA strains in the nasal cavity, mobile phones and pens of students in a university setting in Central Kenya. The findings would then be useful for the comprehension of colonization dynamics within student populations; especially those residing in residence halls within the university, and for the implementation of strategies that can help in preventing the spread and onset of Staphylococcal infections.

CHAPTER THREE

METHODOLOGY

3.1 Study site

The study targeted students residing within the Jomo Kenyatta University of Agriculture and Technology (JKUAT) main campus hostels. This university was selected because no study has previously been done to determine the prevalence of MRSA circulating within the student population. The University admits both male and female students from all over Kenya and around the world and currently has a population of approximately 22000 students in the main campus. Out of 22, 000 students, approximately 6000 students reside within university hostels. Male and female students reside in different hostels but classes are shared (JKUAT Administration, 2015).

3.2 Study design

This was a cross sectional study.

3.3 Study population

The study population comprised of students of JKUAT who reside in the university hostels.

3.4 Criteria for inclusion

i. University students residing within university hostels at the time of recruitment
ii. University students who agreed to provide a written consent for participation and to provide samples.

3.5 Criteria for exclusion

i. Students who were on antibiotics at the time of recruitment.

ii. Students who consented to participate and did not provide a nasal swab.

3.6 Sample size determination

Sample size was calculated using the Cochrane formula (Cochran, 1963).

$$n = \frac{Z^2 pq}{d^2}$$

Where;

n= minimal sample size required for the study.

z= 1.96 (normal deviate corresponding to 95% confidence interval).

d= 0.05 (degree of precision around the mean).

P= 18.8% (represents prevalence of MRSA among primary school children as found in a related study conducted in Jimma town, Ethiopia) (Kejela & Bacha, 2013).

q=1-p

Thus

$$n = \frac{1.96^2 \times 0.188 \times 0.812}{0.05^2}$$

n=235 students

3.7 Recruitment and consenting procedures

Student`s hostel rooms were selected using a systematic random sampling procedure. The student`s rooms were visited during daytime from 10.00AM to 12.00PM for the purpose of recruitment. Students satisfying the selection criteria were selected from the university`s six hostels blocks. In every hostel block, a first room number was picked randomly. This represented the first room from where participants were recruited. The other rooms selected for recruitment were arrived at based on a ratio of the total number of rooms in every hostel block. Hostel blocks F, E and D had 80 student rooms, hostel C and B had 60 student rooms and hostel block A had 700 student rooms, thus the ratio based on a 1:2:3:4:5:6 arrangement was 4:4:4:3:3:35. Therefore, in hostels F, E and D, participants were recruited from every 4^{th} room, in hostels C and B, participants were recruited from every 3^{rd} room and lastly in hostel A, participants were recruited from every 35^{th} room until the minimum required sample size was reached. In hostels F and E, 43 female students were recruited while in hostel C, 32 female students were selected. In hostels D and B, 12 and 8 male students were selected respectively. Lastly, in hostel A, 98 male students were recruited into the study. Every resident student present in the selected hostels at the time of sampling was recruited in the study. Recruitment information sheets (appendix I) containing the objective and methods of this study were issued to participants. The objective and methods were explained to the participants, and thereafter, consent (appendix II) was obtained from the participants prior to enrollment in this study. To ensure participants` privacy, the recruitment process and sample collecting were carried out when doors leading to the rooms were closed. The participants were not requested to provide their names and contacts on the questionnaire that were supplied to them. The questionnaire had unique codes, which served as study identification numbers for all the participants.

3.8 Data collection

Structured questionnaires **(appendix III)** were issued for data collection from consented students. Information collected included sex, age, number of students in sharing a hostel room, the last time the participant visited a hospital for treatment and type of participant`s sporting activity, the behaviour of participants regarding sharing of their items like pens, phones, clothes, sheets as well as the use of medicated soaps while bathing or washing clothes. The questionnaires were administered in quite rooms prior to sample collection.

3.9 Sample collection

To collect a nasal sample, a sterile swab stick was inserted into the nostrils by each participant and gently rotated to scoop a mass of nasal secretions. A single sterile swab was used for each nostril. The sterile swab was pre-moistened with sterile normal saline 0.85% (NaCl) before swabbing. The swabs were labelled using unique codes assigned to each participant before the start of swabbing procedure (Coia *et al.,* 2006).

Single swabs were also collected from mobile phones and pens of participants using sterile swab. The cotton swabs were moistened using sterile normal saline 0.85% (NaCl) and rubbed over the keypads and touch pads of mobiles phones and surfaces of pens. All the surfaces space of targeted surfaces of pens and mobile phones were rubbed for collection of sufficient specimen material. All swabs were put in Amies transport media for transportation to the laboratory at the Centre for Microbiology Research (CMR) and analyzed within a maximum of 4hrs from the time of collection. For enrichment, the swabs were transferred to trypticase soy broth (TSB) and incubated for 18 to 24 hours (Coia *et al.,* 2006).

3.10 Bacterial isolation from nostril, phone and pen swabs

A loopful of inoculum from the TSB cultures was inoculated in on Mannitol Salt Agar (Oxoid) plates and incubated aerobically at 35°C for 24 hours before examination for characteristic colonies of *S. aureus* (Rongpharpi *et al.,* 2013).

3.11 Bacterial identification and preparation of pure colonies

Standard methods of identifying *S. aureus* as described by Baron, (1996) were employed. The plates were assessed for colony morphology and pigment formation associated with *S. aureus.* Typical *S. aureus* colonies showed a yellow pigmentation on Mannitol salt agar. Suspect colonies were subjected to Gram staining, coagulase, and catalase tests (**Appendix IV)**. Pure cultures were prepared and preserved in trypticase soy broth supplemented with 15% glycerol and frozen at -20°C.

3.12 Determination of antimicrobial susceptibility and resistance patterns of *S. aureus* isolates

The modified Kirby-Bauer disk diffusion method used by Rongpharpi *et al*. (2013) was used for the determination of the antibiotic susceptibility patterns. Pure *S. aureus* colonies were suspended in sterile normal saline to make a 0.5 McFarlands turbidity equivalent. A sterile swab was used to inoculate Mueller-Hinton agar plates to obtain an even lawn. Impregnated disks were dispensed equidistant to each other onto the inoculated plates using a commercial dispenser. Commercially available antibiotic discs for Gram positive bacteria were used (Oxoid, CB, UK). The discs included ampicillin (10µg), amoxicillin-clavulanic acid (30), ciprofloxacin (10µg), erythromycin (15µg), gentamicin (10µg), cefoxitin (30µg), linezolid (30µg), norfloxacin (10µg), nitrofurantoin (300µg), chloramphenicol (30µg) and trimethoprim-sulfamethoxazole

(25µg) (CLSI, 2015). *S. aureus* (MRSA) ATCC® 33591 (Oxoid) was used as control. Zones of inhibition were measured after an overnight incubation at 37°C (Rongpharpi *et al.*, 2013). Methicillin-resistant *S. aureus* were detected via the disk diffusion method using cefoxitin (30µg). *S. aureus* isolates resistant to β-lactams and antibiotics from at least three non-β-lactam classes were classified as MDRs. *S. aureus* isolates that displayed zone sizes of less or equal to 21mm were characterized as MRSA while *S. aureus* isolates that displayed zone sizes of more than 21mm were characterized as MSSA (CLSI, 2015).

3.13 Extraction and storage of DNA

The *S. aureus* strains DNA was extracted using 10% Chelex solution prepared using 1X TE buffer following a modification of a procedure used by HwangBo *et al.* (2010). A 300µl volume of 10% Chelex solution was added to fresh sterile Eppendorf tubes. To each Eppendorf tube filled with 300µl volume of 10% Chelex solution, a loopful of 24 hours old bacterial colonies were emulsified. The Chelex/sample tubes were then vortexed for 5-10 seconds after which, the tubes were transferred to a heating block at 95 ºC for 20 minutes. After boiling for 20 minutes, the tubes were left to cool for 10 minutes, before centrifugation at 14700 rpm for 10 minutes. Thereafter, 50µl of the supernatant was transferred to fresh Eppendorf tubes prefilled with 450µl of RNase DNase free PCR water. All the tubes were labelled carefully using the respective isolate codes and stored at -20 ºC for future use.

3.14 Amplification and detection of *Spa* gene

The *Spa* gene encodes for Staphylococcal protein A, an adherence factor, and was expected to be present in all the *S. aureus* isolates. Polymerase chain reaction was used for the amplification of ***Spa*** gene in *S. aureus* isolates. Forward primer 5'- TAA AGA CGA TCC TTC GGT GAG C -3

and reverse primer 5'- CAG CAG TAG TGC CGT TTG CTT -3' (Macrogen, UK) were used to amplify *Spa* gene. In every PCR tube, 4µl of the ready to mix 5x FIREPol®Master Mix (Solis Biodyne, UK), 0.4µl of both forward and reverse primers at a concentration of 10pmol of each primers, An aliquot of 1µl of BSA, 12.2 µl of RNase DNase free PCR water and 2µl of sample DNA. A single control strain of *S. aureus* was incorporated in the DNA amplification process. PCR reactions were set for 5 minutes at 80°C; 35 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 90 seconds at 72°C; a single final extension at 72°C for 10 minutes and a holding temperature at 4°C. The excepted band sizes were to be variable (Strommenger *et al.,* 2008).

3.15 Amplification and detection of *mecA* gene

The *mecA* gene was tested because it is responsible for methicillin resistance among *S. aureus* isolates (Wielders *et al.,* 2002). PCR amplification and detection of *mecA* gene was done on all (231) *S. aureus* isolates using primers listed in **Table 3-1.** In every PCR tube, 4µl of the ready to mix 5x FIREPol®Master Mix (Solis Biodyne, UK), 0.4µl of both forward and reverse primers, 1µl of BSA, 12.2µl of RNase DNase free PCR water and 2µl of sample DNA. Amplification reactions were set at an initial denaturation temperature of 95 ºC for 5 minutes. Thereafter, the preparation was subjected to 30 cycles of denaturation at 94 ºC for 60 seconds, annealing at 56 ºC for 60 seconds, extension at 72 ºC for 60 seconds and final extension at 72 ºC for 7 minutes. A holding temperature was set at 4°C. One control strain was incorporated in the DNA amplification process. The excepted PCR product encoding for *mecA* gene were 533 bp in size (Kondo *et al.,* 2007).

3.16 SCC*mec* typing

SCC*mec* typing was done for all isolates using PCR. Primers specific for SCC*mec*I, SCC*mec*II, SCC*mec*III, SCC*mec*IVa, SCC*mec*IVb, SCC*mec*IVc, SCC*mec*IVd and SCC*mec*V genes were used **(Table 3-1)** as published in a previous study by Zhang et al. (2005). PCR procedures were similar to those for the *mecA* gene but the annealing temperature was set at, 65°C for 45 seconds. A control strain of *S. aureus* known to be positive for the gene was incorporated in all the PCR reactions.

3.17 Detection of *lukFS-PV* gene

The *lukFS-PV* gene encodes for PVL, a virulence factor, in *S. aureus* (Melles *et al.,* 2006). PCR was used for the amplification of *lukFS-PV* gene in a sample of 54 *S. aureus* isolates. Selected isolates included all the 26 MRSA strains and 28 MSSA strains selected randomly to represent those obtained from all halls of residence and from students of different demographics. Primers used for amplification and detection of the *lukFS-PV* gene are listed in **Table 3-1**. PCR procedures were similar to those for the *mecA* gene but the annealing temperature was set at 55 ºC for 30 seconds. A control strain of *S. aureus* known to be positive for the gene was incorporated in all the PCR reactions.

3.18 Repetitive sequence-based (REP)-PCR genotyping

A sample of the *S. aureus* isolates were selected for REP-PCR genotyping. Selected strains were distributed across on halls of residence and were from students of different demographics and exhibited all major types of antimicrobial resistance patterns observed in this study. Typing was done using PCR utilizing the Staphrep primer **(Table 3-1)** at a concentration of 75pmol per 31.5-µl assay. Amplification reactions were done using the Pure Taq (Ready-to-go) PCR beads (GE

Healthcare, U.S.A), 1.5µl of primer, 25µl of RNase DNase free PCR water and 5µl of sample DNA. Thermal cycling parameters were set follows: initial denaturation at 95 for 7 minutes followed by 31 cycles of 94 ºC for 1 minute, annealing at 40 ºC for 1 minute, extension at 65 ºC for 8 minutes and a final extension at 65 ºC for 15 minutes. A holding temperature was set at 10 ºC. The rep-PCR banding patterns were analyzed using GelCompar II (Biomerieux Company, France). A dendogram was generated to enable clustering of the *S. aureus* isolates based on the degrees of similarity.

3.19 Visualization of PCR products

PCR products were visualized under a UV transilluminator on 1.2% agarose gel loaded with ethidium bromide.

Table 3-1: Primers used for amplification of selected genes

Type of primer/gene	Primer sequences		Annealing temperature	Amplicon size	Reference
	Forward primer	Reverse primer			
spa	5' TAA AGA CGA TCC TTC GGT GAG C3'	5' CAG CAG TAG TGC CGT TTG CTT3'	60°C	Variable	(Strommenger *et al.*, 2008)
mecA	5'AAA ATC GAT GGT AAA GGT TGG C3'	5'AGT TCT GCA GTA CCG GAT TTG C3'	56°C	533bp	(Kondo *et al.*, 2007)
SCC*mec*I	5'GCT TTA AAG AGT GTC GTT ACA GG 3'	5'GTT CTC TCA TAG TAT GAC GTC C3'	65°C*, 55°C	613bp	(Zhang *et al.*, 2005)
SCC*mec*II	5' CGT TGA AGA TGA TGA AGC G 3'	5'-CGA AAT TGG TTA ATG GAC C3'	65°C*, 55°C	398bp	(Zhang *et al.*, 2005)
SCC*mec*III	5'CCA TAT TGT GTA CGA TGC G 3'	5' CCT TAG TTG TCG TAA CAG ATC G 3'	65°C*, 55°C	280bp	(Zhang *et al.*, 2005)
SCC*mec*IV a	5'GCC TTA TTC GAA GAA ACC G 3'	5'-CTA CTC TTC TGA AAA GCG TCG 3'	65°C*, 55°C	776bp	(Zhang *et al.*, 2005)
SCC*mec*IV b	5'TCT GGA ATT ACT TCA GCT GC3'	5'AAA CAA TAT TGC TCT CCC TC 3'	65°C*, 55°C	493bp	(Zhang *et al.*, 2005).
SCC*mec*IV c	5'ACA ATA TTT GTA TTA TCG GAG AGC 3'	5'TTG GTA TGA GGT ATT GCT GG 3'	65°C*, 55°C	200bp	(Zhang *et al.*, 2005).
SCC*mec*IV d	5'CTC AAA ATA CGG ACC CCA ATA CA3'	5'TGC TCC AGT AAT TGC TAA AG 3'	65°C*, 55°C	881bp	(Zhang *et al.*, 2005).
SCC*mec*V	5'GAA CAT TGT TAC TTA AAT GAG GG 3'	5' TGA AAG TTG TAC CCT TGA CAC C3'	65°C*, 55°C	325bp	(Zhang *et al.*, 2005).
lukFS-PV	5'ATCATTAGGTAAAATGT CTGGACATGATCCA3'	5'CATCAASTGTATT GGATAGCAAAAGC 3'	55°C	Variable	(Lina *et al.*, 1999).
Rep-PCR*	5'TCGCTCAAAACAACGACACC3'		40°C		(Van Der Zee *et al.*, 1999).

The rep-PCR primer is a single primer. For the SCC*mec* typing primers, two annealing temperatures are given. The first annealing temperature (65°C*) was set for the first 10 cycles, and the second annealing temperature (55°C) was set for the 25 additional cycles

3.20 Data management

3.20.1 Data storage

Data captured included the total number of samples collected for nasal, mobile phone and pen swabs, the total number of isolates of *S. aureus* that were isolated. Additional data included data captured on the questionnaire **(Appendix III).** All data were entered in an excel sheet, which was saved in a password protected computer. An online backup of the data was created using a password protected Dropbox system of the Dropbox Inc, USA.

3.20.2 Data analysis

Statistical analysis of the data was also performed using the SPSS version 20. Specifically, the software was used to calculate proportions of MRSA from the total isolates of *S. aureus*, proportions of MRSA isolated from the nostrils, pens and mobile phones. Chi-square and Fisher exact test were used for analyses of bivariate variables while odds ratios were used for analyses of the association between nasal carriage and various factors captured in the questionnaire. The level of significance was set at 0.05.

3.21 Biosafety

During sample collection, gloves and lab coats were worn. Samples were transported to the laboratory using safe, portable coolers. Upon inoculation of samples on Mannitol salt agar plates, the samples were disposed off in the recommended autoclavable biohazard waste bags after which, they will be autoclaved and disposed completely. Lab coats were disinfected using household bleach solution (sodium hypochlorite) before being reused. Gloves were used once and disposed off in autoclavable biohazard waste bags. All glassware were autoclaved prior to reuse. Inoculations, dispensing of media, preparation of PCR master mix, DNA extraction were

in a hood. All material, reagents and equipment will be labelled as necessary. Working benches were disinfected using household bleach solution or ethanol (30%) at all times, before and after use. Hands were washed in using medicated hand washes in running tap water prior to leaving the laboratory.

3.22 Ethical consideration

An approval to conduct this study was sought from KEMRI's SERU and the ethical approval letter with protocol number KEMRI/SERU/CMR/0024/3123 **(Appendix V)** was awarded). To visit and recruit students in the resident hostels, permission was sought from administration offices of Jomo Kenyatta University of Agriculture and Technology and an approval letter of from the department of medical microbiology was awarded **(Appendix VI)**

CHAPTER FOUR

RESULTS

4.1 General participant characteristics

A total of 237 students were recruited to take part in this study. Of these, 120 (50.6%) were males and 117 (49.4%) were females. Characteristics of participants are provided in **Table 4-1** below. All students were aged between 18 and 30 years. Slightly more than a half (125, 52.7%) of the participants lived in groups of four per room. The rest lived in groups of three or less. Majority of the students (157, 66.2%) did not participate in any sporting activities. Also, majority (223, 94.1%) shared their pens and phones with each other. Slightly more than three quarters of the students (180, 75.9%) reported not to share other personal effects such as clothes, towels and bed sheets **(Table 4-1).**

4.2 Hygiene and clinical history

None of the participants had a recent history of hospitalization. Additionally, a high proportion of the participants (225, 94.9%) did not disinfect their mobile phones and pens. Regarding cleaning of nostrils, majority (214, 90.3%) reported to use handkerchiefs while small proportions (9, 3.8% and 14, 5.9%) used their fingers and other means respectively. More than half (148, 62.4%) of the participants used medicated soaps for hand-washing while the rest used non-medicated soaps **(Table 4-1).**

Table 4-1: Distribution of the study population

Variable	Categories	Frequency (n) (N=237)	Percentage
Gender	Males	120	50.6
	Females	117	49.4
Age	18-30 years	237	100
Resident halls	Hall F	48	20.3
	Hall E	39	16.5
	Hall D	13	5.5
	Hall C	30	12.7
	Hall B	9	3.8
	Hall A	98	41.4
Students per room	In Fours	125	52.7
	In Threes	11	4.6
	In Twos	102	43
Last time of hospitalization	Less than 48 hours ago	0	0
	More than 48 hours ago	237	100
Sporting activities	Participated	80	33.8
	Did not participate	157	66.2
Disinfection of pens and phones	Disinfected	12	5.1
	Did not disinfect	225	94.9
Sharing of phones pens	Shared	223	94.1
	Did not share	14	5.9
Sharing of personal effects	Shared	57	24.1
	Did not share	180	75.9
Cleaning of nostrils	Used handkerchief	214	90.3
	Used fingers	9	3.8
	Used other means	8	5.9
Washing hands	Used medicated soaps	148	62.4
	Used non-medicated soaps	89	37.6

Personal effects, things owned and used by students like clothes, towels and bed sheets

4.3 Frequency of isolation of *S. aureus* based on sample analysed

Overall, a large proportion of the participants 87 (36.7%) harboured *S. aureus* at least on their mobile phones compared to the proportions, which carried the bacteria at least on nostrils, 78 (32.9%), and pens, 66 (27.9%) **(Table 4-2).**

Table 4-2: Number of *S. aureus* isolates recovered from nostrils and fomites

Type of sample/s analysed	Number of *S. aureus* isolates recovered from the sample (N=231)	Frequency (%)
Nostril	78	32.91
Phone	87	36.7
Pen	66	27.8
Nostril + Phone	40	16.9
Nostril + Pen	29	12.2
Phone + Pen	31	13.1
Nostril + Phone + Pen	17	7.2

4.4 Nasal colonization and fomite contamination based on participant's gender

Isolation of *S. aureus* from specimens collected from nostrils, phones and pens was analyzed based on the gender of participants. Nasal carriage was significantly higher in females 47 (40.2%) compared to males 31 (25.8%) **(Table 4-3) (**p=0.02, OR=0.52, CI=0.29-0.93) **(Appendix VII).** Of the 120 male participants, 38 (31.7%) harboured *S. aureus* on their mobile phones and 30 (25%) had contaminated pens. In contrast, 49 (41.9%) of the 117 female participants tested positive for phone contamination and 36 (30.8%) for pen contamination. Analysis of carriage of *S. aureus* on multiple specimens revealed that only a small proportion of males, 9 (7.5%), and females, 8 (3.4%), harboured these bacteria on a combination of nostrils,

phones and pens and these carriages were not associated with gender (p value > 0.05) **(Appendix VII).**

4.5 Nasal colonization and fomite contamination based on the halls of residence and mode of occupancy

Prevalence of *S. aureus* was determined based on the halls of residence of the participants and the type of occupancy in the respective hostels. Results indicated that Hall C had a large proportion of its occupants 17 (56.7%) carrying *S. aureus* at least on their nostrils compared to the other halls where carriage ranged from 21(21.4%) – 7 (53.8%). The type of occupancy in Hall C was four students per room. Results also indicated that nasal carriage of *S. aureus* was significantly high among those who stayed in groups of four, 52 (41.9%) (p=0.003, OR= 2.36, CI=1.28 - 4.39) **(Appendix VII)** compared to those who stayed in groups of three, 3 (27.3%), and two, 23 (22.5%). The study also established the existence of a higher levels of *S. aureus* contamination of phones used by students occupying Hall E, 20 (51.3%) compared to other halls where carriage was at lower percentages of between 4 (44.4%) and 29 (29.6%). Similar to the case of nostril colonization, students in this hall stayed in fours, and its participants also had a high carriage of *S. aureus* on phones, 49 (39.5%), compared to those who stayed in groups of three, 4 (36.4%), and two 32 (31.4%). A large proportion of the participants, 17 (35.4%), carrying the bacteria on their pens resided in Hall F while the least proportion, 3 (23.1%), resided in Hall D. Regarding the mode of occupancy, those who stayed in fours (the case for Hall F had a high carriage, 37 (29.8%), of *S. aureus* on the pens compared to those who stayed three, 3 (27.3%), and two 25 (25.5%) students per hostel room. Analysis of carriage of *S. aureus* on multiple sites showed that less than 25% of participants from all the hostels carried *S. aureus* in their nostrils, phones and pens at the same time. Additionally, less than 10% of students in each

of the three modes of occupancy were found to harbour the bacteria in their nostrils, phones and pens at ago **(Table 4-3).** Based on these results, it was established that nasal colonization varied significantly across the six hostels (p= 0.008). However, contamination on fomites did not vary significantly between halls (p value > 0.05) **(Appendix VII).**

4.6 Nasal colonization and fomite contamination based on sports

Analysis of students' participation in sports was done, coupled with selected variables to establish possible associations with colonization and contamination by *S. aureus*. Overall, the study showed that a large proportion of students who participated in sporting activities, 30 (37.5%), carried the bacteria on their phones compared to the counterpart group where two large proportions, each 57 (36.3%), were found to carry *S. aureus* at least on their nostrils or phones **(Table 4-3).** In general, participation in sports was not associated with colonization and contamination by *S. aureus* (p>0.05) **(Appendix VII).**

Further, analysis showed that compared to female students, male students who participated in sports were more likely to test positive for nasal colonization (p= 0.03, OR= 3.05, CI= 0.99-9.59), their phones were also likely to be contaminated (p= 0.003, OR= 4.33, CI= 1.54-12.48) as well as their pens (p= 0.001, OR= 7.09, CI= 1.91-27.91) **(Appendix VIII).** Analysis also showed that among students who participated in sporting activities, those who lived in congested conditions (four students per room) were more likely to harbour the bacteria in their nostrils (p= 0.05, OR= 0.37, CI= 0.12-1.14), phones (p= 0.002, OR= 0.23, CI= 0.08-0.65), and pens (p= 0.01, OR= 0.25, CI= 0.07-0.85) compared to those who stayed in less four per room **(Appendix IX).**

4.7 Nasal colonization and fomite contamination on sharing items

Results showed that among students who shared their phones and pens, a high proportion, 84 (37.7%), harbored *S. aureus* at least on phones compared to the counterpart group where high proportions, 4 (28.6%), carried S. aureus on nostrils or pens. Large proportions of students, 23 (40.4%), who shared their personal effects and those who did not, 64 (35.6%), carried the bacteria at least on their phones **(Table 4-4).**

No statistical significance was established between carriage of *S. aureus* and the sharing behaviours among students (p value >0.05) **(Appendix IX).**

4.8 Nasal colonization and fomite contamination based on hygienic practices

Colonization and contamination by *S. aureus* was analyzed based on several hygienic practices. These included; disinfection of phones and pens, nostril cleaning and hand-washing. Results indicated that slightly more than a third of the participants, 80 (36.4%), who cleaned their nostrils using handkerchiefs harboured *S. aureus* on their phones. It was also noted that only students who used handkerchiefs to clean their nostrils harboured *S. aureus* in all the sites analysed (nostrils, phones and pens). Among the 9 (3.8%) students who cleaned their nostrils using fingers, a third, 3 (33.3%), harboured *S. aureus* at least in their nostrils or phones. A similar proportion, 3 (37.5%), of 8 (5.9%) students who cleaned their noses using other means carried *S. aureus* at least on their nostrils or phones. Phone contamination was noted in 59 (39.9%) of students who use medicated soaps to clean their hands while 28 (31.5%) of students who used non-medicated soaps to clean their hands had their phones contaminated. Regarding the 89 (37.6%) students who did not wash their hands using medicated soaps, a high carriage, 31 (34.8%), was found on nostrils compared to the carriage, 48(32.4%) observed for in counterpart group. In all the cases of hygienic practices, least proportions (less than 10% for all the cases)

were found to be colonized on their nostrils and had contaminated phones and pens **(Table 4-4).** Additional analysis showed that associations between respective hygienic practices and colonization by *S. aureus* in any of the single or multiple sites were statistically insignificant ($p>0.05$) **(Appendix X).**

Table 4-3: Proportions of students who harboured *S. aureus* based on gender, residency and sporting

Variable	Categories	Number of participants (N)	Colonization and contamination on a single site			Colonization and contamination on multiple sites			
			Nostril, n (%)	Phone, n (%)	Pens, n (%)	Nostrils and phone n (%)	Nostril and pen n (%)	Phone and pen n (%)	All sites n (%)
Gender	Males	120	31 (26.7)	38 (31.7)	30 (25)	15 (12.5)	14 (11.7)	14 (11.7)	9 (7.5)
	Females	117	47 (40.2)	49 (41.8)	36 (30.8)	25 (21.4)	15 (12.8)	17 (14.5)	8 (3.4)
Resident halls	Hall F	48	15 (31.1)	16 (33.3)	17 (35.4)	6 (12.5)	4 (8.3)	6 (12.5)	1 (2.1)
	Hall E	39	15 (38.5)	20 (51.3)	10 (25.6)	10 (25.6)	6 (15.4)	7 (17.9)	4 (10.3)
	Hall D	13	7 (53.8)	5 (38.5)	3 (23.1)	2 (15.4)	1 (7.7)	2 (15.4)	1 (7.7)
	Hall C	30	17 (56.7)	12 (40)	9 (30)	9 (30)	4 (13.3)	4 (13.3)	3 (10)
	Hall B	9	3 (33.3)	4 (44.4)	3 (33.3)	2 (22.2)	2 (22.2)	2 (22.2)	2 (22.2)
	Hall A	98	21 (21.4)	29 (29.6)	24 (24.5)	12 (12.2)	11 (11.2)	10 (10.2)	6 (6.1)
Occupancy	In Fours	124	52 (41.9)	49 (39.5)	37 (29.8)	25 (20.2)	17 (13.7)	20 (16.1)	11 (8.9)
	In Threes	11	3 (27.3)	4 (36.4)	3 (27.3)	4 (36.4)	0	0	0
	In Twos	102	23 (22.5)	32 (31.4)	26 (25.5)	12 (11.8)	12 (11.8)	11 (10.8)	6 (5.9)
Sporting activities	Participated	80	22 (27.5)	30 (37.5)	21 (26.3)	12 (15)	11 (13.8)	12 (15)	8 (10)
	Did not participate	157	57 (36.3)	57 (36.3)	45 (28.7)	29 (18.5)	18 (11.5)	19 (12.1)	9 (5.7)

Table 4-4: Proportions of students who harboured *S. aureus* based on hygiene practices

Variable	Categories	Number of participants (N)	Colonization and contamination on a single site			Colonization and contamination on multiple sites			
			Nostril, n(%)	Phone, n(%)	Pens, n(%)	Nostrils and phone n(%)	Nostril and pen n(%)	Phone and pen n(%)	All sites n(%)
Disinfection of pens and phones	Disinfected	12	4 (33.3)	4 (33.3)	3 (25)	1 (8.3)	1 (8.3)	1 (8.3)	0
	Did not disinfect	225	75 (33.3)	83 (36.9)	63 (28)	40 (17.8)	28 (12.4)	30 (13.3)	17 (7.6)
Sharing of phones/pens	Shared	223	75 (33.6)	84 (37.7)	62 (27.8)	40 (17.9)	26 (11.7)	30 (13.5)	16 (7.2)
	Did not share	14	4 (28.6)	3 (21.4)	4 (28.6)	1 (7.1)	3 (21.4)	1 (7.1)	1 (7.1)
Sharing of personal effects	Shared	57	20 (35.1)	23 (40.4)	14 (24.6)	14 (24.6)	5 (8.8)	7 (12.3)	4 (7)
	Did not share	180	59 (32.8)	64 (35.6)	52 (31.7)	27 (15)	24 (13.3)	24 (13.3)	13 (7.2)
Cleaning of nostrils	Used handkerchief	220	72 (32.7)	80 (36.4)	63 (28.6)	38 (17.3)	29 (13.2)	28 (12.7)	17 (7.7)
	Used fingers	9	3 (33.3)	3 (33.3)	1 (11.1)	2 (22.2)	0	1 (11.1)	0
	Used other means	8	3 (37.5)	3 (37.5)	2 (25)	1 (12.5)	0	2 (25)	0
Washing hands	Used medicated soaps	148	48 (32.4)	59 (39.9)	43 (29.1)	29 (19.6)	19 (12.8)	22 (14.9)	13 (8.8)
	Used non-medicated soaps	89	31 (34.8)	28 (31.5)	23 (25.8)	12 (13.5)	10 (11.2)	9 (10.1)	4 (4.5)

*Personal effects, things owned and used by students like clothes, towels and bed sheet

4.9 Antimicrobial resistance profiles of *S. aureus* isolates recovered students

A total of 231 isolates recovered from the nostrils, phones and pens were tested against 11 antimicrobials. A marked ampicillin-resistance, 194 (84%), was observed among these isolates. Resistances to other drugs were less than 20%. Resistance prevalences to cefoxitin and trimethoprim-sulfamethoxazole were over 10% while resistances to amoxicillin, chloramphenicol, gentamicin, ciprofloxacin, nitrofurantoin and norfloxacin were below 10%. None of the isolates were resistant to linezolid and only a single case of intermediate resistance was observed for nitrofurantoin. Based on the AST results, 26 out of the 231 *S. aureus* isolates were cefoxitin-resistant making the prevalence of MRSA (cefoxitin-resistant isolates) in this study to be recorded at 11.3% **(Figure 4-1).**

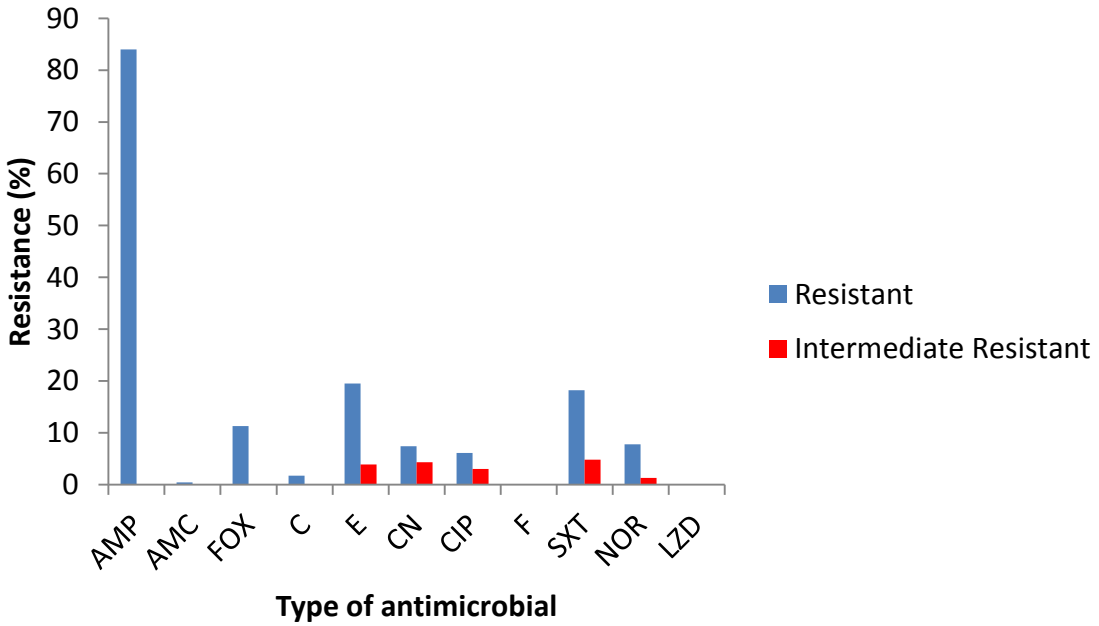


Figure 4-1: Overall percent resistances of *S. aureus* isolates from the sampled students.

***AMP**- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid.

4.9.1 Antimicrobial resistance profiles based on gender

Results from this study indicated that resistance to ampicillin was high (over 75%) in isolates recovered from both males, 80 (80.8%), and females, 114 (87%). Amoxicillin-clavulanic acid resistance was detected in a single isolate recovered from a female participant who resided in Hall C. Among isolates recovered from females, percent resistances to the other antimicrobials were less than 21 % with the exception of nitrofurantoin and linezolid where none of the *S. aureus* isolates from females showed resistance. None of the isolates recovered from males showed resistance to either amoxicillin or linezolid. A single case of intermediate resistance to nitrofurantoin was observed in an isolate recovered from a male residing in Hall A and a single case of gentamicin resistance was recorded in an isolate recovered from a male participant living in Hall D. Among the *S. aureus* recovered from males, percent resistances to the other antimicrobials such as cefoxitin, chloramphenicol, erythromycin, ciprofloxacin, norfloxacin and trimethoprim-sulfamethoxazole ranged from 2 (2%) in chloramphenicol to 17 (17.2%) in trimethoprim-sulfamethoxazole **(Figure 4-2).** Isolates recovered from females were more likely to be resistant to erythromycin (P= 0.03, OR= 0.44= CI= 0.19-1.01) **(Appendix XI)** compared to isolates recovered from males. It was also established that *S. aureus* that isolates recovered from males were more likely to be resistant to trimethoprim-sulfamethoxazole (p= 0.020, OR= 1.62= CI= 0.71-3.65) **(Appendix XI)** compared to isolates recovered from females.

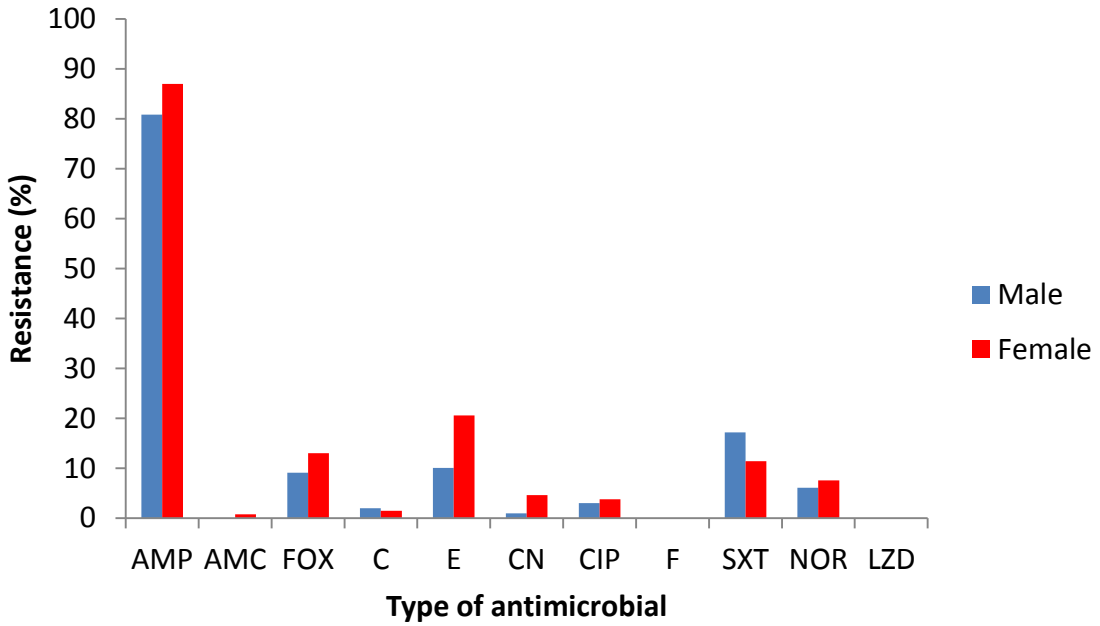


Figure 4-2: Resistance prevalence with respect to gender.

***AMP**- ampicillin, **AMC**- amoxicillin, **FOX**- Cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid.

4.9.2 Antimicrobial resistance profiles based on the halls of residence

Antimicrobial resistance prevalence were analyzed based on the halls of residence from which the *S. aureus* were recovered. Resistance to ampicillin was more than 75% across all residence halls. A single case of amoxicillin-clavulanic acid resistance was observed in isolate recovered from Hall C. In addition, isolates recovered from students residing in Hall C had resistance prevalences of 21.1% to cefoxitin, 23.7% to erythromycin, 23.5% to trimethoprim-sulfamethoxazole and 10.5% to norfloxacin compared to other halls where lower resistance prevalences to the respective antimicrobials were recorded **(Figure 4-3).**

The differences in resistance levels recorded for trimethoprim-sulfamethoxazole across the six residence halls were statistically significant (p value = 0.02) **(Appendix XI).**

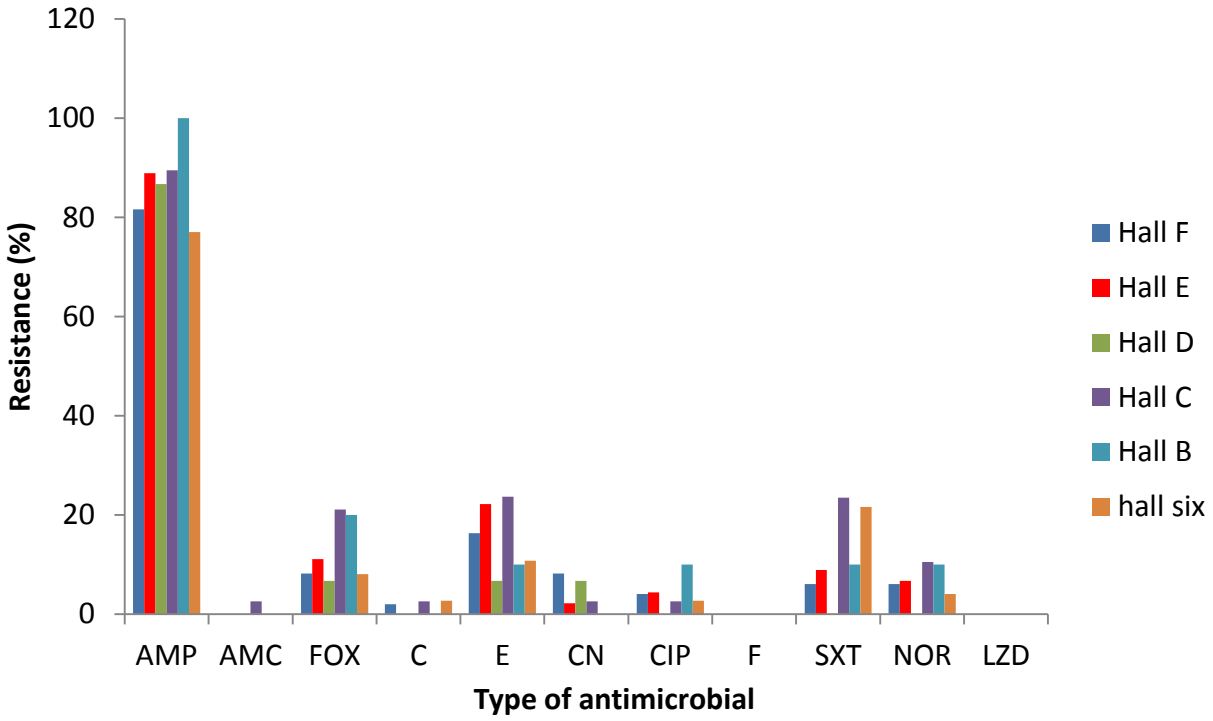


Figure 4-3: Resistance prevalence based on residence halls.

> ***AMP**- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid.

4.9.3 Antimicrobial resistance profiles based on the modes of occupancy

Resistance profiles of isolates recovered from participants were analyzed based on the modes of occupancy adopted by participants. Consequently, more than 75% of *S. aureus* isolates showed ampicillin-resistance regardless of the mode of occupancy (staying in groups of two, three of four). A single case of amoxicillin-clavulanic acid-resistance was recorded in an isolate recovered from a participant who reported to stay four students in their hostel room. Chloramphenicol resistances were also low, with cases of resistance being recorded among *S. aureus* isolates recovered from those staying in twos, 2 (1.3%), and fours, 2 (1.4%) only. At least 2 (16.7%) of isolates recovered from students staying in threes showed resistance to

ciprofloxacin, compared to isolates recovered from those staying in twos and fours where resistance levels were 2 (2.5%) and 4 (2.9%) respectively. Similarly, resistance to norfloxacin was also high among isolates recovered from students who stayed in threes, 2 (16.7%), compared to those who stayed in twos and fours where resistance levels were found to be 5 (6.3%) and 9 (6.5%) respectively. It was also observed that students who stayed in twos had a high trimethoprim-sulfamethoxazole resistance, 16 (20%), compared to those who stayed in fours 14 (10.1%) and threes 2 (16.7%). Resistance to erythromycin was found to be high among students who stayed in threes, 5 (41.7%), compared to students who stayed in twos, 8 (10%), and fours 24 (17.3%). Gentamicin-resistance was also high, 2 (16.7%), among isolates recovered from participants who stayed in threes compared to isolates recovered from those who stayed in fours 4 (3.6%). No case of gentamicin resistance was recorded among isolates recovered from students who stayed in twos **(Figure 4-4).** This study, however, established that there was no statistically significant association between the type of accommodation and antimicrobial resistance to like ampicillin, amoxicillin , cefoxitin, chloramphenicol , ciprofloxacin and trimethoprim-sulfamethoxazole ($P>0.05$). On the contrary, isolates recovered from students staying in threes were more likely to be resistant to erythromycin (p value = 0.03) and gentamicin (p value = 0.01), compared to isolates recovered from participants who stayed in twos and fours **(Appendix XI).**

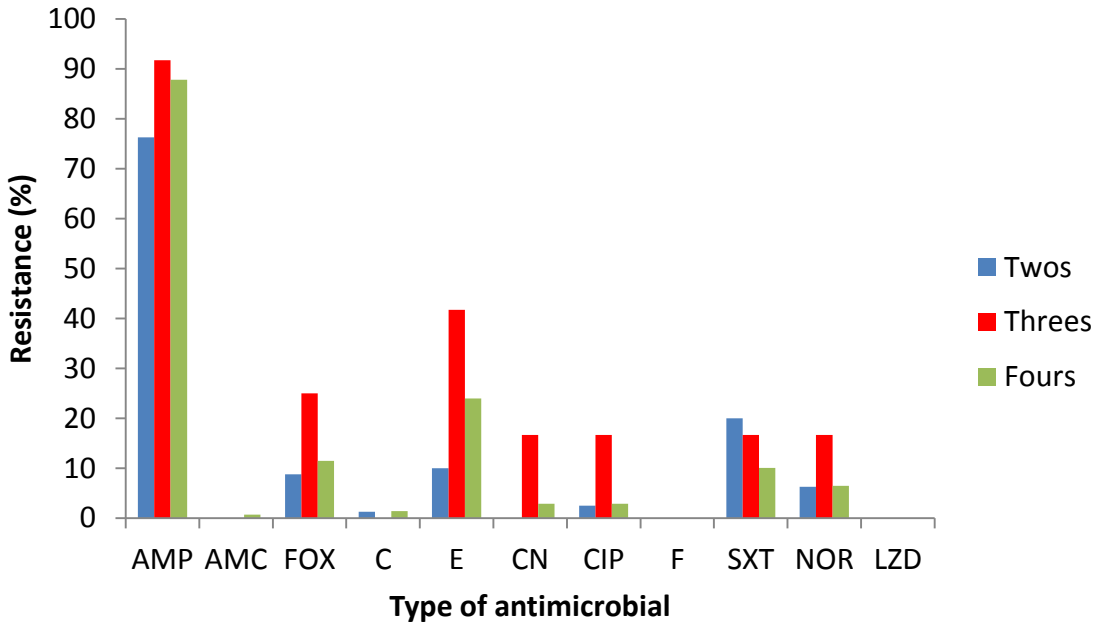


Figure 4-4: Resistance prevalence based on the mode of occupancy.

***AMP**- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**-chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**-nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**-linezolid. **Twos**- students stay two per room, **Threes**-students staying three per room, **Fours**-students staying four per room. **Twos**- students staying 2 per room, **Threes**-students staying 3 per room, **Fours**- students staying 4 per room

4.9.4 Antimicrobial resistance profiles based on sports

Antimicrobial resistance profiles of the *S. aureus* isolates were analyzed based on the participants' participation in sports. Accordingly, high levels of resistance to ampicillin (over 75%) were found among isolates recovered from the students who participated and those who did not participate in sports. Resistance to amoxicillin-clavulanic acid was low, 1 (1.4%), with the single case of resistance being recorded in an isolate recovered from a student who participated in sports. It was also noted that resistance to cefoxitin, 9 (12.2%), ciprofloxacin, 3 (4.1%), trimethoprim-sulfamethoxazole, 16 (21.6%) and norfloxacin, 6 (8.1%), were higher among isolates recovered from students who participated in sports compared to resistance levels

recorded for the respective antimicrobials among isolates recovered from the counterpart group. In contrast, isolates recovered from students who did not participate in sports had slightly higher resistances to chloramphenicol, 3 (1.9%), and gentamicin, 5 (3.2%), compared to isolates recovered from students who participated in sports where resistances were recorded in 4 (1.4%) and 7 (2.7%) of the *S. aureus* isolates respectively **(Figure 4-5).** No statistically significant associations were found between sports and resistance to amoxicillin-clavulanic acid, cefoxitin, chloramphenicol, erythromycin, gentamicin, ciprofloxacin and norfloxacin ($p>0.05$) **(Appendix XI).**

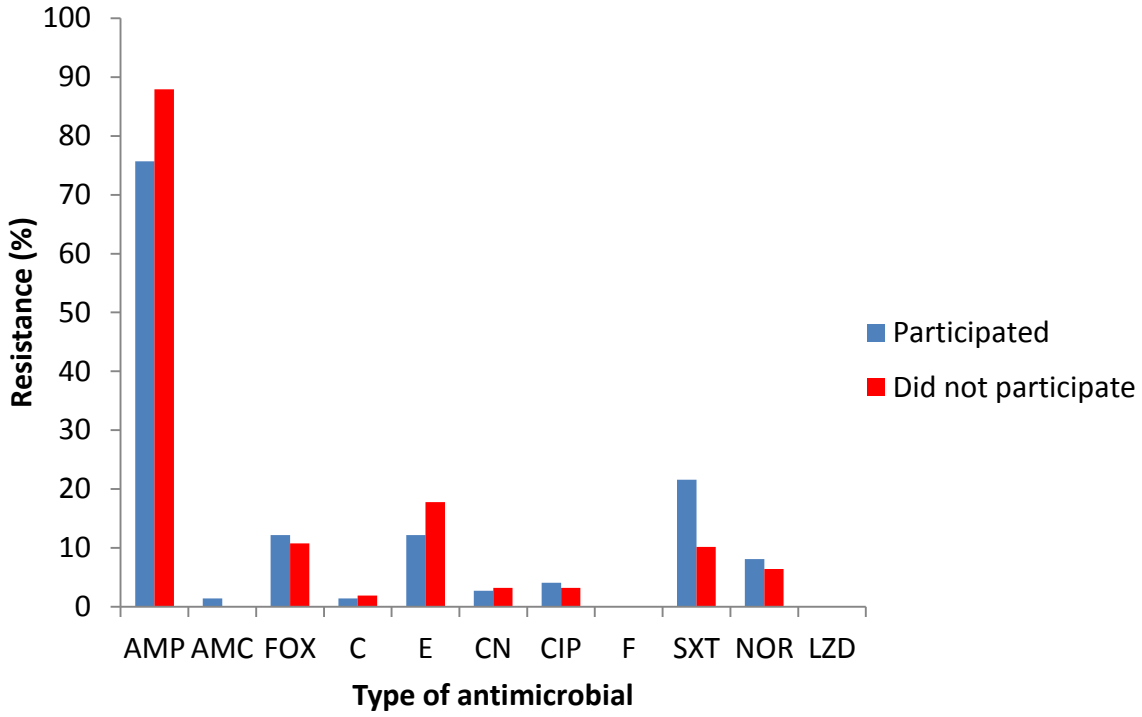


Figure 4-5: Resistance prevalence based on sports.

***AMP-** ampicillin, **AMC-** amoxicillin-clavulanic acid, **FOX-** cefoxitin, **C-** chloramphenicol, **E-** erythromycin, **CN-** gentamicin, **CIP-** ciprofloxacin, F- nitrofurantoin, **SXT-** trimethoprim-sulfamethoxazole, **NOR-** norfloxacin, **LZD-** linezolid.

4.9.5 Antimicrobial resistance profiles based on the practice of disinfecting phones and pens

Antimicrobial resistance profiles of the *S. aureus* isolates were analyzed based on the participants` behaviours of disinfecting or not disinfecting their phones and pens. High levels of resistance to ampicillin of were recorded among isolates recovered from those who disinfected 10 (100%) and those who did not disinfect their phones and pens 184 (83.3%). Resistance to amoxicillin-clavulanic acid was recorded in a single isolate recovered from a participant who did not disinfect her phones or the pens. It was also noted that resistances to cefoxitin, 4 (40%), chloramphenicol, 2 (20%), erythromycin, 2 (20%) and gentamicin, 3 (30%) were high among isolates recovered from students who disinfected their phones and pens compared to respective resistances recorded in isolates recovered from students who reported not to disinfect their phones and pens. Resistance to ciprofloxacin, trimethoprim-sulfamethoxazole and norfloxacin were recorded in 8 (3.6%), 32 (14.5%) and 16 (7.2%) of isolates recovered from students who did not disinfect their phones and pens **(Figure 4-6).** Additional analysis revealed that disinfecting phones and pens was significantly associated with resistance to cefoxitin (p= 0.01, OR= 6.03, CI= 1.30-26.85), chloramphenicol (p= 0.01, OR= 27.38, CI= 2.35-328.87) and gentamicin (p= 0.002, OR= 23.25, CI= 3.31-164.54) **(Appendix XII).**

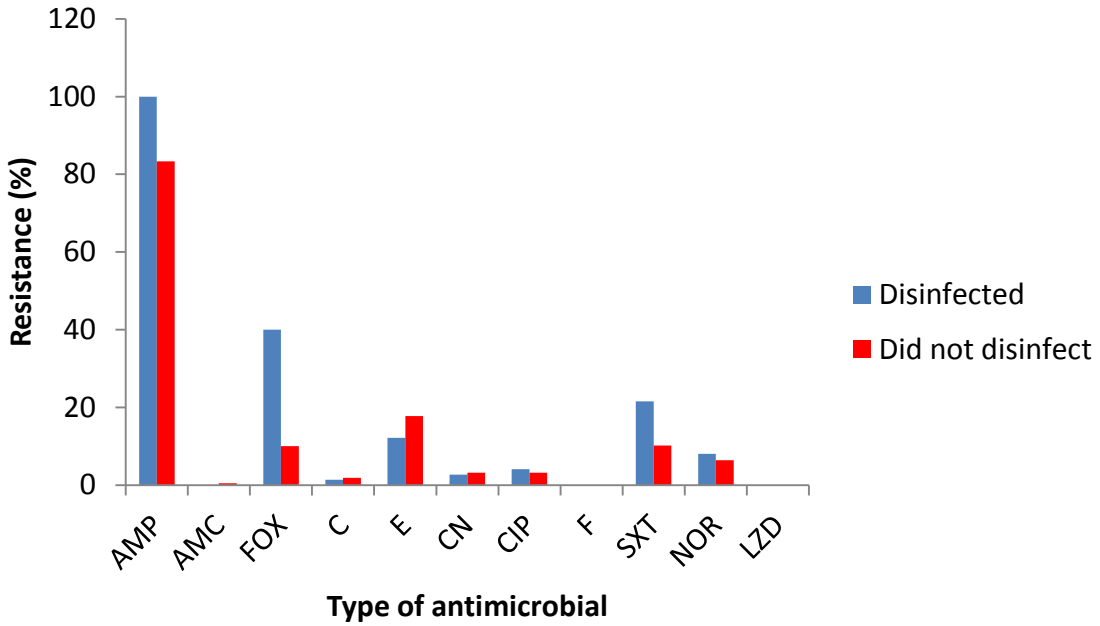


Figure 4-6: Resistance prevalences based on the practice of disinfecting phones and pens.

***AMP-** ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**-chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**-nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**-linezolid.

4.9.6 Antimicrobial resistance profiles based on the behaviour of sharing phones and pens

Analysis of antimicrobial resistance profiles of the *S. aureus* isolates was a done based on the participants` behaviour of sharing their phones and pens. Percent resistance to ampicillin was more 80% among isolates recovered from the two categories of students. Resistance to cefoxitin, 25 (11.4%) and erythromycin, 36 (16.4%), were high among isolates recovered from participants who shared their phones and pens compared to the counterpart group where equal proportions (both 9.1%) of the isolates showed resistance to the respective antimicrobials. Resistance to amoxicillin, 1 (0.5%), ciprofloxacin, 8 (3.6%), trimethoprim-sulfamethoxazole, 32 (14.5%), and norfloxacin, 16 (7.3%), were recorded among students who shared their phones and pens only **(Figure 4-7).** This study revealed that isolates recovered from students who shared their phones

and pens were more likely to be resistant to gentamicin (p= 0.04, OR= 0.11, CI= 0.01-0.91) compared to isolates obtained from the counterpart group **(Appendix XII).**

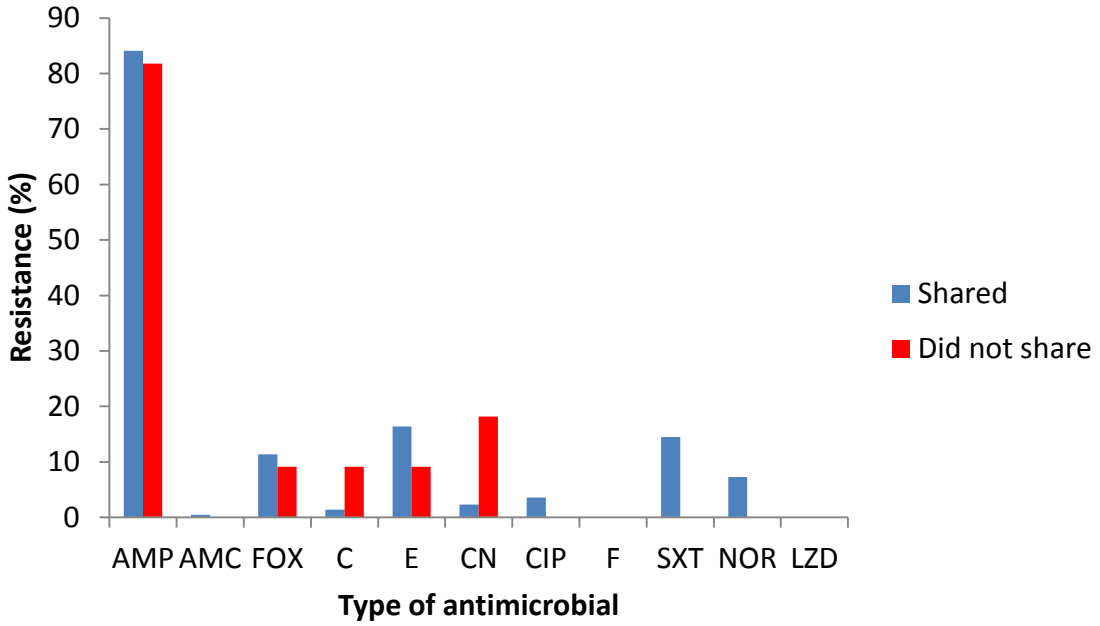


Figure 4-7: Resistance prevalence based on the behaviour of sharing phones and pens.

***AMP**- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid

4.9.7 Antimicrobial resistance profiles based on the behaviour of sharing personal effects

Analysis of antimicrobial resistance profiles of the *S. aureus* isolates was done based on the participants` behaviour of sharing their personal effects. These included items like towels, bed sheets or clothes. Analysis showed that resistance to ampicillin was high with proportions of resistant isolates obtained from students who shared their personal effects and those who did not share their personal effects being, 52 (91.2%) and 142 (81.6)% respectively. Resistance to amoxicillin was recorded in a single isolate recovered from a female student who reported to share her personal effects. Resistance to chloramphenicol was also low, 4 (2.3%), and was only recovered among isolates recovered from participants who did not share their personal effects.

Resistances to cefoxitin, 7 (12.4%), erythromycin, 10 (17.5%), trimethoprim-sulfamethoxazole, 11 (19.3%), and norfloxacin, 4 (7%) were high among isolates recovered from students who shared their personal effects compared to resistance levels, 19 (10.9%), 27 (15.5%), 21 (12.1%), and 12 (6.9%), observed among isolates recovered from the counterpart group **(Figure 4-8).** Further analysis showed that isolates recovered from participants who shared their personal effects were more likely to be resistant to trimethoprim-sulfamethoxazole (p= 0.01, OR= 3.54, CI= 1.33-9.49) **(Appendix XII)** compared to other antimicrobials.

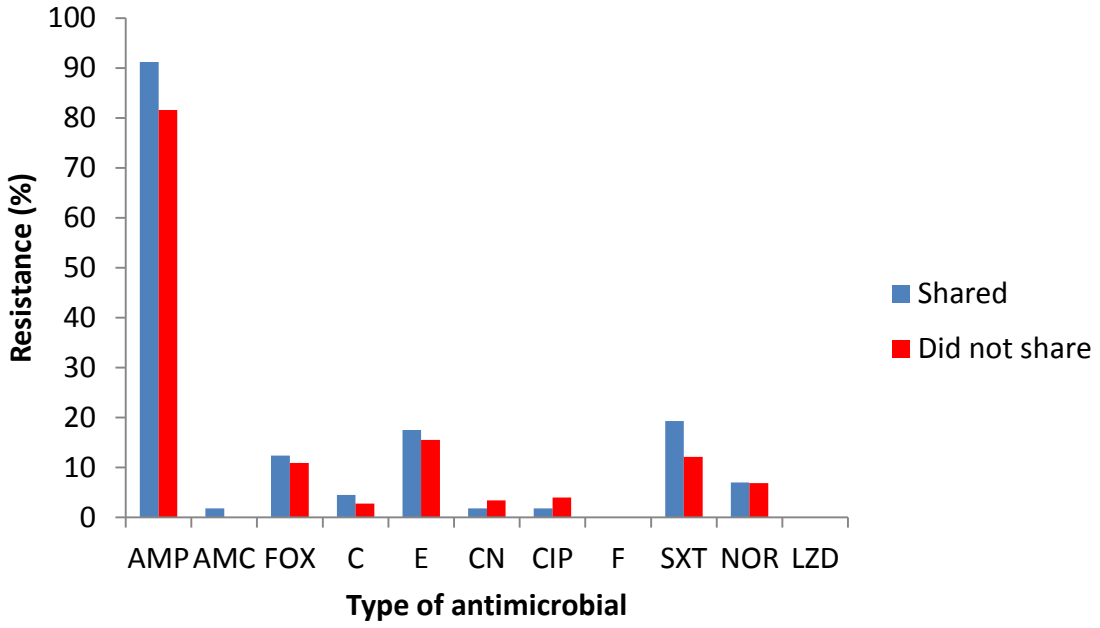


Figure 4-8: **Prevalence of resistance based on the behaviours of sharing personal effects.**

***AMP**- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, F- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid

4.9.8 Antimicrobial resistance profiles based on the means of cleaning nostrils

The prevalence of antimicrobial resistance among the *S. aureus* isolates recovered from participants in this study was analyzed based on the participants` practice of cleaning their nostrils using handkerchiefs, fingers and other means such as use of clothes, sheets or towels.

Analysis revealed the existence of a high prevalence of resistance to ampicillin among isolates recovered from students who reported to clean their nostrils using handkerchiefs, 184 (85.6%), and those who reported to use other means 7 (70%). For participants who used fingers to clean their nostrils, only 3 (50%) of the isolates recovered showed resistance to ampicillin. Also a similar proportion of isolates in this group (those who used fingers) was resistant to erythromycin. A single case of amoxicillin-clavulanic acid resistance was also recorded among students who used handkerchiefs to clean their nostrils. Resistance to chloramphenicol, 4 (1.9%), was only noted among participants who used handkerchiefs to clean their nostrils. High resistance prevalences to cefoxitin, 2 (20%), gentamicin, 1 (10%), ciprofloxacin, 1 (10%), and norfloxacin, 1 (10%) were also observed among isolates recovered from participants who cleaned their nostrils using other means compared to the resistance prevalences recorded for cefoxitin, 24 (11.2%), gentamicin, 6 (2.8%), ciprofloxacin, 7 (3.3%), and norfloxacin, 15 (7%) recorded among isolates recovered from participants who used handkerchiefs to clean their nostrils. No resistance to cefoxitin, gentamicin, ciprofloxacin and norfloxacin was recorded in isolates obtained from participants who used fingers to clean their nostrils. It was also noted that isolates recovered from students who used fingers to clean their nostrils showed higher resistance levels to trimethoprim-sulfamethoxazole, 1 (16.7%), compared to resistance levels, 1 (10%) and, 30 (14%), recorded among isolates from participants who used other means and fingers respectively **(Figure 4-9).** Further analysis demonstrated that isolates recovered from students who used handkerchiefs to clean their nostrils were more likely to be resistant to ampicillin (p value = 0.02) and erythromycin (p value = 0.001) compared to other antimicrobials **(Appendix XII).**

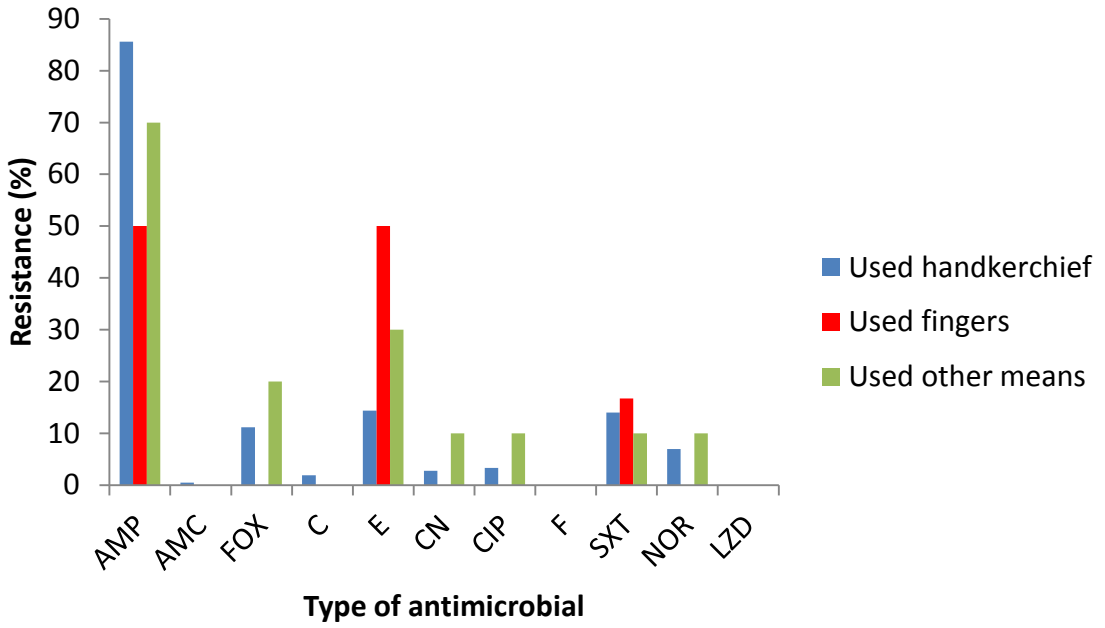


Figure 4-9: Resistance prevalence based on the means of cleaning nostrils.

***AMP**- ampicillin, **AMC**-amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**-chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**-nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid

4.9.9 Antimicrobial resistance profiles based on the type of soap used for handwashing

The antimicrobial resistance profiles of the isolates was analysed based on the students behaviour of using medicated and non medicated soaps. The prevalence of ampicillin resistance was high among participants who used medicated 133 (89.3%) and those who used non-medicated soaps 61 (74.4%). The single strain that exhibited resistance to amoxicillin-clavulanic acid was also recovered from a participant who used medicated soap. Resistance to gentamicin was observed in 7 (4.7%) isolates obtained from students who used medicated soaps. Resistance prevalences to cefoxitin, 21 (14.1%), chloramphenicol, 3 (2%), erythromycin, 26 (17.4%), ciprofloxacin, 6 (4%), trimethoprim-sulfamethoxazole, 22 (14.8%) and norfloxacin, 13 (8.7%) were observed to be high among isolates recovered from students who used medicated soaps compared to the respective prevalences recorded for isolates recovered from students who used

non medicated soaps with resistances where resistance levels were recorded as follows; cefoxitin, 5 (6.1%), chloramphenicol, 1 (1.2%), erythromycin, 11 (13.4%), ciprofloxacin, 2 (2.4%), trimethoprim-sulfamethoxazole, 10 (12.2%), and norfloxacin, 3 (3.7%) **(Figure 4-10).** Based on these results, the study showed that ampicillin and gentamicin-resistance were highly likely to be associated with the use of medicated soaps (p values = 0.003 and 0.046 respectively). On the contrary, no significant association was established between the use of medicated or non-medicated soap and resistance to amoxicillin, cefoxitin, chloramphenicol, erythromycin, ciprofloxacin, trimethoprim-sulfamethoxazole and norfloxacin (p>0.05) **(Appendix XII).**

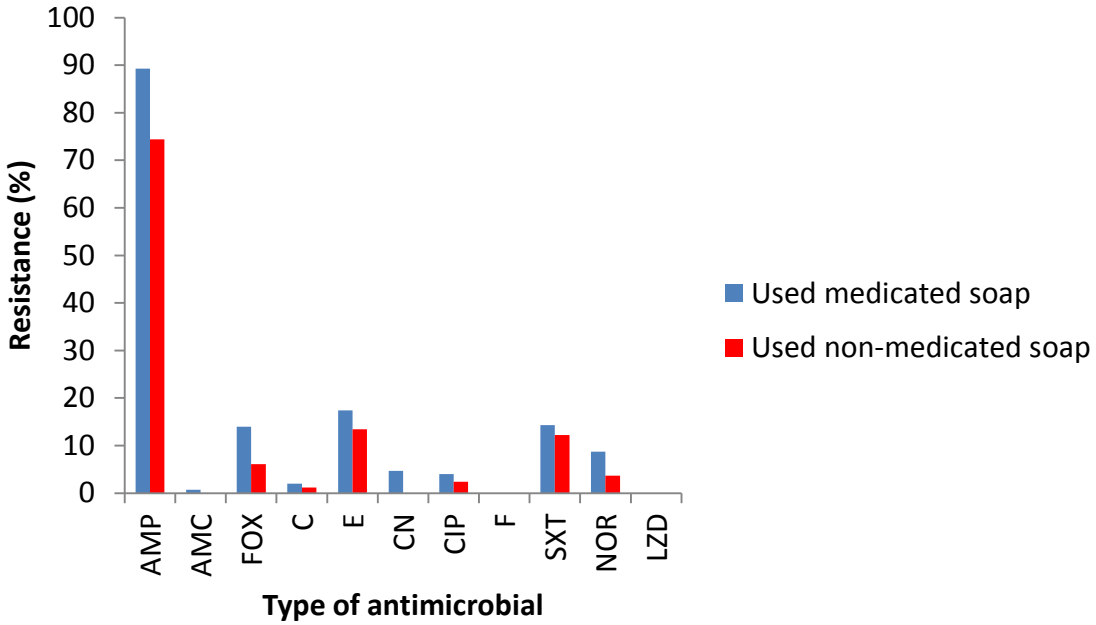


Figure 4-10: Resistance prevalence based on the means of cleaning nostrils.

***AMP**- ampicillin, **AMC** amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid

4.10 Resistance phenotypes associated with MRSA and MDR strains

Based on the antimicrobial susceptibility screening of the 231 isolates, 26 (11.3%) were found to be MRSA while 16 (9.9%) were MDRs. Out of the 26 MRSA strains, 13 (50%) were also

MDRs. Overall, this study established that all the MRSA and MDR strains were resistant to ampicillin compared to only 80.5% of the MSSA strains. In addition, resistances above 40% were observed for erythromycin, norfloxacin and trimethoprim-sulfamethoxazole among the MRSAs. Similar observations were made for the MDRs, which also showed over 40% resistance levels to other antimicrobials like cefoxitin (81.3%), ciprofloxacin (50%) and norfloxacin (75%). For both MRSA and MDRs, less than 20% resistance levels were recorded for amoxicillin-clavulanic acid, chloramphenicol and gentamicin. Less than 20% of the MSSA strains were resistant to the all the antimicrobials except ampicillin while a majority of the MRSAs (57.7%) and MDRs (75%) were found to be resistant to erythromycin **(Figure 4-11).** This study also established that MRSA strains were more likely to display multi-drug resistance compared MSSA strains (P value = 0.001).

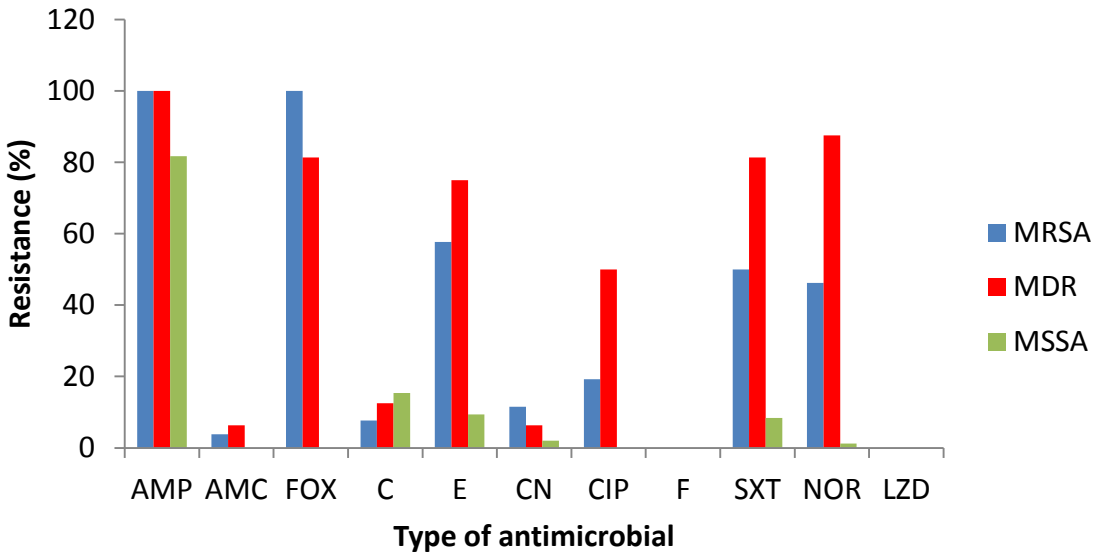


Figure 4-11: Antimicrobial resistance patterns among the MRSA, MDR and MSSA strains.

***AMP**- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid, **MRSA**- methicillin-resistant *S. aureus*, **MDR**- multi-drug resistant, **MSSA**- methicillin-susceptible *S. aureus.*

4.11 Multi-drug resistance patterns in *S. aureus* isolates recovered from the JKUAT student population

The *S. aureus* recovered from participants showed multiple patterns of multidrug resistance. A large proportion of MDRs, 4 (1.73%), was resistant to six antimicrobials in the following pattern; AMP/FOX/E/CIP/SXT/NOR followed by, 3 (1.3%), which was also resisted six antimicrobials in the following pattern; AMP/FOX/C/E/SXT/NOR. Nine resistance patterns of four, five and six antimicrobials were observed in equal proportions, 1 (0.43%), of the isolates. The maximum number of antimicrobials resisted by MDR strains was six antimicrobials. The respective MDR patterns included; AMP/FOX/E/CIP/SXT/NOR, AMP/AMC/FOX/CIP/SXT/NOR and AMP/FOX/C/E/SXT/NOR. On the other hand, the minimum number of antimicrobials resisted by the MDR strains was four antimicrobials with MDR patterns of AMP/E/CIP/SXT and AMP/E/CIP/NOR **(Table 4-5).**

Table 4-5: Multi-drug resistance patterns of *S. aureus* isolates

Resistance panel	MDR pattern	Number of resistant strains (N=231) n (%)
4 antimicrobials	AMP/E/CIP/SXT	1 (0.43%)
	AMP/E/CIP/NOR	1 (0.43%)
5 antimicrobials	AMP/FOX/C/E/CN	1 (0.43%)
	AMP/FOX/E/SXT/NOR	1 (0.43%)
	AMP/FOX/CIP/SXT/NOR	1 (0.43%)
	AMP/FOX/CN/CIP/NOR	1 (0.43%)
	AMP/FOX/E/SXT/NOR	3 (1.30%)
	AMP/E/CIP/SXT/NOR	1 (0.43%)
6 antimicrobials	AMP/FOX/E/CIP/SXT/NOR	4 (1.73%)
	AMP/AMC/FOX/CIP/SXT/NOR	1 (0.43%)
	AMP/FOX/C/E/SXT/NOR	1 (0.43%)

AMP- ampicillin, **AMC**- amoxicillin-clavulanic acid, **FOX**- cefoxitin, **C**- chloramphenicol, **E**- erythromycin, **CN**- gentamicin, **CIP**- ciprofloxacin, **F**- nitrofurantoin, **SXT**- trimethoprim-sulfamethoxazole, **NOR**- norfloxacin, **LZD**- linezolid

4.12 Prevalence of *spa, mecA* and *LukFS-PV* genes among the *S. aureus* isolates

All the *S. aureus* isolates recovered in this study were screened for the presence of *spa* and *mecA* genes. For the *lukFS-PV* gene, only a sample of the isolates was selected as described previously. Overall, all the *S. aureus* isolates were found to carry the *spa* gene. Presence of the *mecA* gene was detected in only 17 (7.4%) of all the *S. aureus* isolates. However, with respect to the MRSA and MDR strains, the prevalence of *mecA* gene was high among the MRSA strains, 17 (65.4%), compared to the MDRs, 9 (56.3%). The MSSA strains did not harbour the *mecA* gene. Less than 15% of isolates recovered from the nostrils and fomites carried *mecA* gene **(Table 4-6).**

Table 4-6: Proportion of *S. aureus* isolates harbouring the *spa* and *mecA* genes

Type/source of isolate	Number of isolates tested (n)	Type of gene, n (%)	
		spa	***mecA***
Overall	231	231 (100%)	17 (7.4%)
MRSA	26	26 (100%)	17 (65.4%)
MSSA	205	205 (100%)	0 (0%)
MDR	16	16 (100%)	9 (56.3%)
Nostrils	78	78 (100%)	8 (10.3%)
Fomites	153	153 (100%)	9 (5.9%)

MRSA- methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall**- all isolates that were selected to screen for the presence of the *lukFS-PV* gene

Regarding the *lukFS-PV* gene, the overall prevalence was low, with slightly more than a quarter, 17 (31.5%), of the sampled isolates carrying this gene. It was also noted that a large proportion of the MSSA, 10 (35.7%), carried the *lukFS-PV* gene, compared to the proportion of MRSAs, 7 (26.9%), and MDRs, 4 (25%)**.** Further analysis revealed the lack of a significant difference between the MSSA and MRSA strains (*p* value = 0.487) **(appendix XII)** with regard to the carriage of the *lukFS-PV* gene. Basing on the sites from which the isolates were recovered, this study established that slightly more than a quarter of the sampled isolates from the nostrils, 7 (31.8%), and fomites, 10 (31.3%), carried the *lukFS-PV* gene **(Table 4-7).** Photographed gel profiles for the *spa*, *lukFS-PV* and *mecA* genes are shown in **figure 4-23**.

Table 4-7: Proportion of *S. aureus* isolates harbouring the *lukFS-PV* genes among the sampled *S. aureus* isolates

Type/source of isolate	Number of *S. aureus* isolates screened (n)	*lukFS-PV* gene, n (%)
Overall	54	17 (31.5%)
MRSA	26	7 (26.9%)
MSSA	28	10 (35.7)
MDR	16	4 (25%)
Nostril	22	7 (31.8%)
Fomites	32	10 (31.3%)

MRSA- methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall**- all isolates that were selected to screen for the presence of the *lukFS-PV* gene

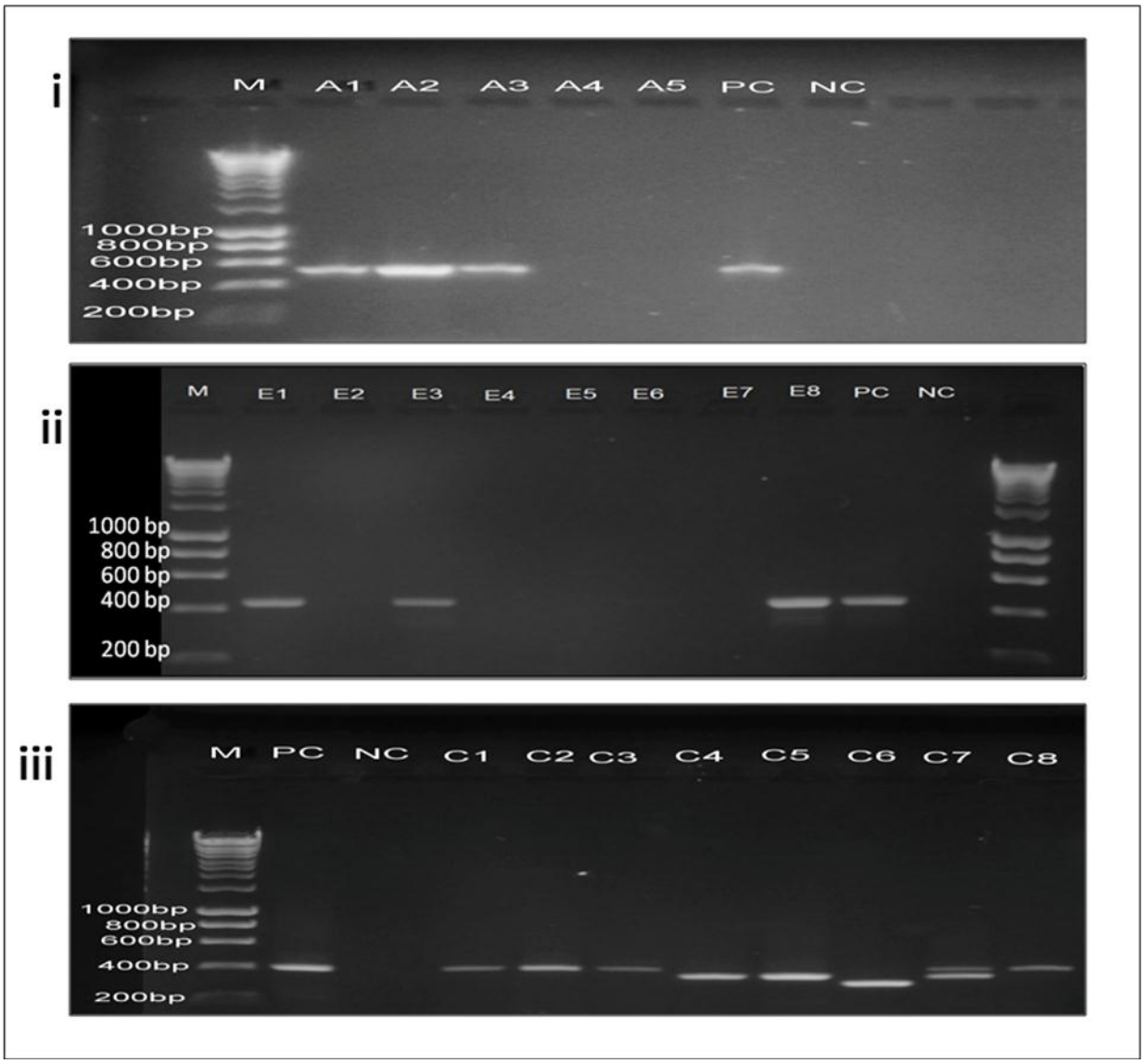


Figure 4-12: Electrophoresis gel results for *mecA, Spa* and *lukFS-PV* genes

*__i__ - *mecA* gene (533bp), **ii** - *lukFS-PV* genes (433BP), **iii** - *spa* gene (variable band sizes)

4.13 Prevalence of SCC*mec* elements among the *S. aureus* isolates

All the *S. aureus* isolates were screened for SCC*mec* elements included in this study. Overall, the prevalence of various *SCCmec* types were; SCC*mec*I, 7 (3%), SCC*mec*II, 15 (6.5%), SCC*mec*III, 3 (1.3%), and SCC*mec*V, 16 (6.9%), but SCC*mec*IVa, *SCCmec*IVb, *SCCmec*IVc and SCC*mec*IVd were absent in these isolates**.** The prevalence of SCC*mec*I was high among the

MRSA strains, 4 (15.4%), compared to the prevalence in MDR strains, 1 (6.3%), and MSSA strains, 2 (1%). Small proportions (less than 5%) of isolates recovered from the nostrils and fomites were found to also harbour *SCCmec*I. The prevalence of SCC*mec*II was found to be high among the MDRs, 11 (68.8%), than in MRSA strains, 14 (53.9%) and the MSSA, which did not harbour SCC*mec*II. A large proportion of the isolates from the nostrils, 10 (12.8%), also harboured SCC*mec*I compared to the proportion of isolates recovered from fomites, 5 (3.3%). SCC*mec*III was only present in, 3 (11%) of the MRSA isolates and in only, 2 (2.6%), and, 1 (0.7%) of the isolates recovered from the nostril and fomites respectively. Similar to SCC*mec*II, the prevalence of SCC*mec*V was found to be slightly higher among the MDRs, 10 (62.5%), compared to MRSAs, 16 (61.5%) **(Table 4-8).** Photographed gel profiles of the SCC*mec* elements are shown in **figure 4-24**.

Table 4-8: Proportion of *S. aureus* isolates carrying SCC*mec* elements

Type/source of isolate	Number of isolates tested (n)	Type of SCC*mec* element, n (%)			
		I	**II**	**III**	**V**
Overall	231	7 (3%)	15 (6.5%)	3 (1.3%)	16 (6.9%)
MRSA	26	4(15.4%)	14 (53.9%)	3 (11.5%)	16 (61.5%)
MSSA	205	2 (1.0%)	0 (0%)	0 (0%)	0 (0%)
MDR	16	1 (6.3%)	11 (68.8%)	0 (0%)	10 (62.5%)
Nostril	78	3 (3.9%)	10 (12.8%)	2 (2.6%)	10 (12.8%)
Fomites	153	4 (2.6%)	5 (3.3%)	1 (0.7%)	6 (3.9%)

The prevalences for SCC*mec*IVa, SCC*mec*IVb, SCC*mec*IVc and SCC*mec*IVd were 0%, and therefore, were not included in the table; **MRSA-** methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall**- all isolates that were selected to screen for the presence of the *lukFS-PV* gene

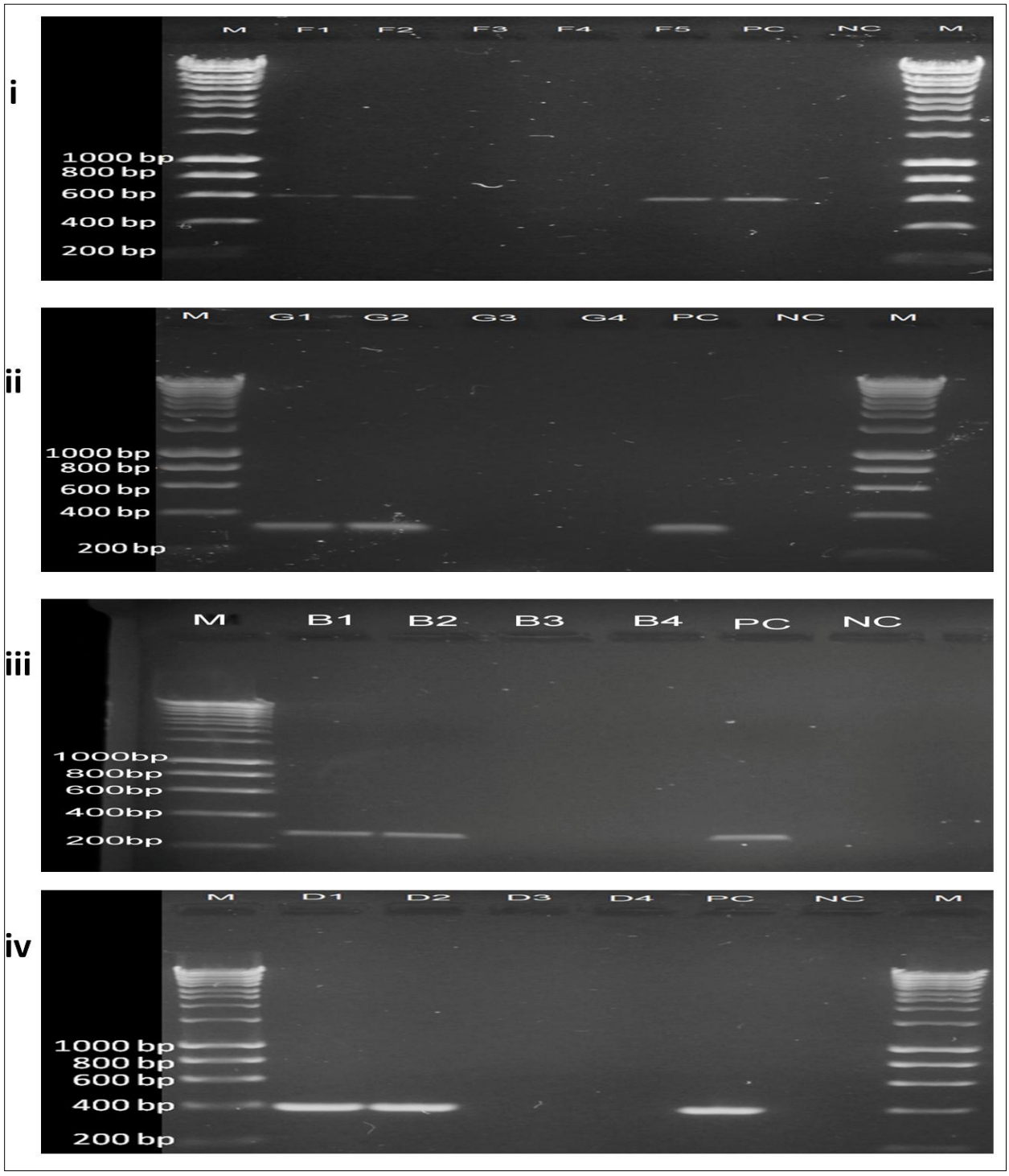


Figure 4-13: Electrophoresis gel results for SCC*mec* elements

*__i__- SCC*mec*I (613bp), __ii__- SCC*mec*II (398bp), __iii__- SCC*mec*III (280bp), __iv__- SCC*mec*V (325bp)

4.14 Prevalence of SCC*mec* elements, unidentified SCC*mec* elements and *lukFS-PV* genes elements among the *mecA*-positive isolates

All the *mecA-positive* strains were screened for the presence of selected SCC*mec* elements and *lukFS-PV* genes in their gene cassettes. Accordingly, SCC*mec*V was found to be highly prevalent, 11 (64.7%), compared to SCC*mec*II, 8 (47.1%), SCC*mec*I, 5 (29.4%), and SCC*mec* III, 2 (11.8%) among the *mecA*-positive MRSA strains. Contrary to the *mecA*-positive MRSA strains, the SCC*mec*II were harboured in the majority, 7 (77.8%), of the *mecA*-positive MDR strains. A majority of *mecA*-positive isolates recovered from the nostrils, 6 (75%), and fomites, 5 (55.6%), were also found to harbour SCC*mec*V. The unidentified SCC*mec* elements were found in small proportions of the *mecA*-positive MRSA strains, 3 (17.6%), and the *mecA*-positive isolates from the nostrils, 3 (37.5%) **(Table 4-9).**

The prevalence of the *lukFS-PV* genes among the *mecA*-positive MRSA and *mecA*-positive MDR strains was, 5 (29.5%) and 3 (33.3%) respectively**.** The study also established that carriage of the *lukFS-PV* genes was, 3 (37.5%) and 2 (22.2%) among *mecA*-positive isolates recovered from the nostrils and fomites respectively **(Table 4-9).**

Table 4-9: Proportion of *mecA*-positive *S. aureus* isolates carrying SCC*mec* elements, unidentified SCC*mec* elements and *lukFS-PV*

Type/source of isolate	Number of *mecA*-positive isolates (N)	SCC*mec* type, n(%)				Unidentified SCC*mec* elements, n(%)	*lukFS-PV* genes, n (%)
		I	II	III	V		
MRSA	17	5 (29.4%)	8 (47.1%)	2 (11.8%)	11 (64.7%)	3 (17.6%)	5 (29.5%)
MDR	9	1 (11.1%)	7 (77.8%)	0	3 (33.3%)	0 (0%)	3 (33.3%)
Nostril	8	3 (37.5%)	5 (62.5%)	1 (12.5%)	6 (75%)	0 (0%)	3 (37.5%)
Fomites	9	2 (22.2%)	3 (33.3%)	1 (11.1%)	5 (55.6%)	3 (33.3%)	2 (22.2%)

MRSA- methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall**- all isolates that were selected to screen for the presence of the *lukFS-PV* gene

4.15 Prevalence of SCC*mec* elements and *lukFS-PV* genes elements among the *mecA*-negative isolates

Overall, the *mecA*-negative isolates formed a large proportion (92.6%) of the isolates recovered from participants. These isolates were also screened for the presence SCC*mec* elements including SCC*mec* I, II, III, IVa, IVb, IVc, IVd and V. The isolates were also screened of *lukFS-PV* genes. In general, small proportions of *mecA*-negative isolates harboured SCC*mec*I, 2 (0.9%), SCC*mec*II, 7 (3.3%), SCC*mec* III, 1 (0.5%), and SCC*mec*V, 5 (2.3%). The majority of the *mecA*-negative MRSA isolates harboured SCC*mec*II, 6 (66.7%), and SCC*mec*V, 5 (55.6%), compared to SCC*mec*III, 1 (11.1%). The SCC*mec*I was the only gene detected in 2 (1.0%) of the *mecA*-negative MSSA strains. The SCC*mec*II and SCC*mec*V were present in 7 (57.1%), and 2 (28.6%) of the *mecA*-negative MDR strains respectively. Small proportions of *mecA*-negative isolates recovered from nostrils (less than 10%) were found to harbour SCC*mec*I, II, III and V while those recovered from fomites harboured SCC*mec*I, II and V **(Table 4-10).** Close to a third of the sampled *mecA*-negative isolates were found to harbour the *lukFS-PV* genes, 12 (32.4%). Almost a quarter of the *mecA*-negative MRSA isolates, 2 (22.2%), and, 1 (16.67%), of the *mecA*-

negative MDR strains carried the *lukFS-PV* gene in their gene cassettes. On the other hand, less than 35% of both *mecA*-negative isolates recovered from nostrils and fomites carried this gene **(Table 4-11).**

Table 4-10: Proportion of *mecA* negative *S. aureus* isolates carrying the SCC*mec* elements

Type/source of isolate	Number of *mecA*-negative isolates (N)	SCC*mec* type, n (%)			
		I	II	III	V
Overall	214	2 (0.9%)	7 (3.3%)	1 (0.5%)	5 (2.3%)
MRSA	9	0 (0%)	6 (66.7%)	1 (11.1%)	5 (55.6%)
MSSA	202	2 (1.0%)	0 (0%)	0 (0%)	0 (0%)
MDR	7	0 (0%)	4 (57.1%)	0 (0%)	2 (28.6%)
Nostril	70	5 (7.1%)	1 (1.4%)	1 (1.4%)	4 (5.7%)
Fomites	144	2 (1.4%)	2 (1.4%)	0 (0%)	1 (0.7%)

MRSA- methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall**- all isolates that were selected to screen for the presence of the *lukFS-PV* gene

Table 4-11: Proportion of *mecA*-negative *S. aureus* isolates harbouring *lukFS-PV* genes

Type/source of isolate	Number of isolates tested	*lukFS-PV* gene, n (%)
Overall	37	12 (32.4%)
MRSA	9	2 (22.2%)
MSSA	28	10 (35.7%)
MDR	6	1 (16.6%)
Nostril	14	4 (28.5%)
Fomites	23	8 (34.7%)

MRSA- methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall**- all isolates that were selected to screen for the presence of the *lukFS-PV* gene.

4.16 Prevalence of *S. aureus* isolates with multiple SCC*mec* elements

An assessment for the presence of multiple SCC*mec* elements within the gene cassettes of isolates recovered in this study was done. Based on this assessment, four different combinations, each of two distinct SCC*mec* elements were evident. These included; SCC*mec*I and SCC*mec*III, SCC*mec*I and SCC*mec*V, SCC*mec*II and SCC*mec*V and SCC*mec*II and SCC*mec*V. Analysis revealed that less than 5% of the isolates recovered in this study harboured multiple SCC*mec* elements with the SCC*mec*II and SCC*mec*V combination appearing in 4.8% of the isolates. Almost half of the MRSA strains, 11 (42.3%), carried SCC*mec*II and SCC*mec*V compared to those that carried SCC*mec*I and SCC*mec*V, 3 (11.5%), SCC*mec*I and SCC*mec*III, 2 (7.7%), and SCC*mec*III and SCC*mec*V, 1 (3.9%). Only two combinations of the SCC*mec* elements were seen among the MDR strains where, 8 (50%) of these strains carried SCC*mec*II and SCC*mec*V and only a single MDR varied SCC*mec*I and SCC*mec*V. All the combinations were evident among isolates recovered from the nostrils with, 7 (9%) of these isolates harbouring both SCC*mec*II and SCC*mec*V. Each of the other combinations was found in less than 3% of the isolates recovered from the nostrils. Isolates recovered from the fomites displayed on three combinations including SCC*mec*I and SCC*mec*III, 1 (0.7%), SCC*mec*I and SCC*mec*V, 1 (0.7%) and SCC*mec*II and SCC*mec*V, 4 (2.6%) **(Table 4-12).**

Table 4-12: Proportion of *S. aureus* isolates carrying multiple SCC*mec* elements

Type/ source of isolate	Number of *S. aureus* isolates tested (N)	SCC*mec* elements combination, n (%)			
		SCC*mec*I + SCC*mec*III	**SCC*mec*I + SCC*mec*V**	**SCC*mec*II + SCC*mec*V**	**SCC*mec*III + SCC*mec*V**
Overall	231	2 (0.9%)	3 (1.3%)	11 (4.8%)	1 (0.4%)
MRSA	26	2 (7.7%)	3 (11.5%)	11 (42.3%)	1 (3.9%)
MDR	16	0 (0%)	1 (6.3%)	8 (50%)	0 (0%)
Nostril	78	1 (1.3%)	2 (2.6%)	7 (9.0%)	1 (1.3%)
Fomites	153	1 (0.7%)	1 (0.7%)	4 (2.6%)	0 (0%)

MRSA- methicillin-resistant *S. aureus,* **MDR-** multi-drug resistant, **MSSA-** methicillin-susceptible *S. aureus,* **Overall-** all isolates that were selected to screen for the presence of the *lukFS-PV* gene

4.17 Genetic relationships of *S. aureus* isolates recovered from the student population

Rep-PCR successfully differentiated the *S. aureus* isolates recovered from the participants. **Figure 4-26** represents a dendogram that included selected isolate profiles on the basis of the level of their similarities. The selected isolate profiles included; the place of isolation, gender of participant from which the isolate was obtained, the hall of residence, number of students in a room occupied by the participant, whether the participant participated or did not participate in sports, the type of strain of the isolate and presence or absence of the *mecA* gene in the gene cassettes of respective isolates. Based on a 40% level of similarity, the *S. aureus* isolates clustered in 10 different clusters identified as Cluster A to J in **Figure 4-26**. Cluster A was composed of 9 (22.5%) of the selected isolates, most of which were recovered from females with only a single isolate recovered from a male participant. Isolates from females circulated within all the female residence halls included in this study. These isolates were a mixture of MDR,

MSSA and MDR/MRSA strains with many of them, 7 (77.5%), being MRSA. Some of the isolates, 6 (66.7%), harboured *mecA* gene in their gene cassettes, while others, 3 (33.3%), did not. Despite being recovered from male and female participants, the two isolates in cluster B were both MRSA strains and harboured *mecA* gene. Possibly, these isolates clustered together since they MRSA strain types and carried the *mecA* gene. Isolates in cluster C, sub-cluster C2 were a mixture of both MRSA and MSSA strains, but were found not to harbour *mecA* gene in their gene cassettes. Cluster E was also composed of two MSSA strains that tested negative for *mecA* and were recovered from male participants residing in the same residence hall. Cluster F isolates circulated in three different halls occupied by both male and female participants. Possibly, these isolates appeared to cluster together because they were both MSSA strains and also lacked *mecA* gene. All isolates belonging to cluster I were recovered from nostrils and were also MSSA strain types. Generally, it was evident that the individual isolate profiles had a role to play in the clustering of the respective *S. aureus* isolates **(Figure 4-26).**

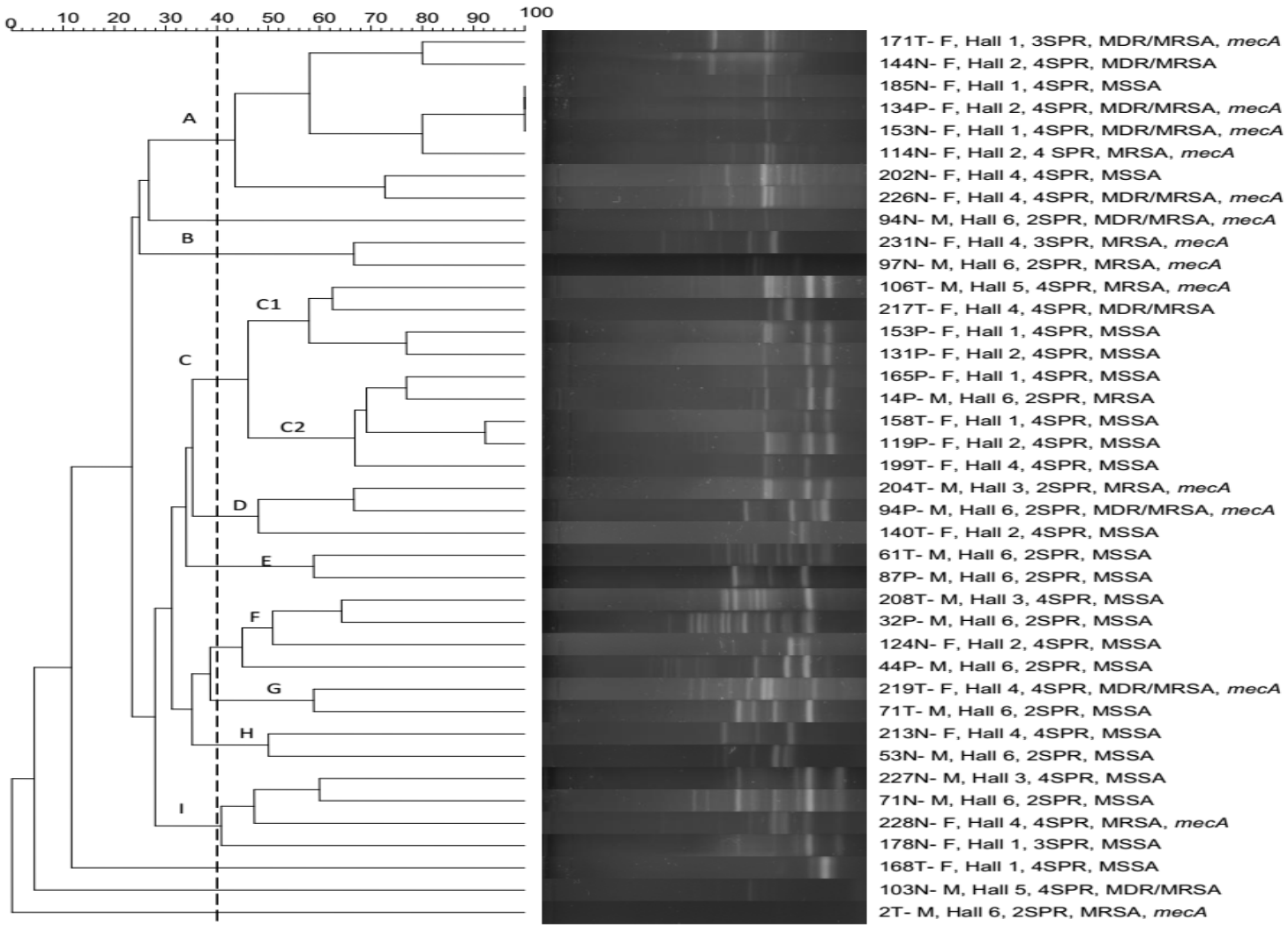


Figure 4-14: Rep-PCR-generated dendogram for the 40 *S. aureus* isolates from the student population.

* A 40% level of similarity was used for the interpretation of the relatedness of the isolates as depicted by the vertical line.
The nine clusters are designated A to I. F- female, M- male, SPR- number of students per room. MDR/MRSA- multi-drug resistant/methicillin-resistant *S. aureus*, MSSA- methicillin-susceptible *S. aureus*.

4.18 Analysis of factors associated with MRSA and MDR colonization and contamination among the sampled students

Analysis of possible factors associated with the colonization by MRSA strains was done. Based on this analysis, the study established that there was no significant association between gender and colonization by MRSAs (p= 0.37, OR= 0.68, CI= 0.26-1.70). Regarding the halls of residence, this study established that isolates recovered from Halls E, C and B were more likely to be MRSAs (p= 0.03, OR= 2.46, CI= 0.98-6.25) as opposed to isolates recovered from Hall F, three and six. No significant association was established between the mode of occupancy and colonization by MRSAs (p= 0.38, OR= 0.67, CI= 0.24-1.78). Additionally, no significant association was found between participation in sporting activities and colonization by MRSAs (p= 0.77, OR= 1.14, CI= 0.442-2.89) **(Appendix XIV**). Failure to disinfect mobile phones and pens was also found to be significantly associated with colonization of MRSAs (p= 0.02, OR= 6.03, CI= 1.30-26.86). Colonization by MDRs was also found to be significantly associated with sharing of personal effects (p= 0.001, OR= 0.02, CI= 0.003-0.084) **(Appendix XV).** Based on these findings, it was speculated that staying in Halls E, C and B and failing to disinfect mobile phones and pens were factors for MRSA colonization while sharing of personal effects was a factor for MDR colonization.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of *S. aureus* colonization

Carriage of *S. aureus* in a population that is otherwise considered as healthy has shown global variation. Previous studies have revealed colonization prevalences ranging between 20%-40%, which is reported as the prevalence limit in literature, in different study populations (Chambers & Deleo, 2009; Sivaraman *et al.*, 2009). In this study, the prevalence of nasal, phone and pen colonization fell within the range of 20-40% **(Table 4-2)**. Similar results have also been reported by other studies which focused on college students in Tanzania (Okamo *et al.*, 2016), China (Du *et al.*, 2011), Nepal (Ansari *et al.*, 2016) as well as the general population in Nigeria (Onanuga & Temedie, 2011), Gabon (Schaumburg *et al.*, 2011), Brazil (Pires *et al.*, 2014), Northern Manhattan (M. Miller et al., 2009) and Mexico (Hamdan-Partida *et al.*, 2010). On the contrary, other published reports have indicated prevalences outside the 20-40% limit. A study conducted on healthy volunteers in Tunisia reported a nasal colonization prevalence of 13% (Ben Slama *et al.*, 2011) while another study focused on a remote population in Guiana reported a colonization prevalence of 57.8% (Ruimy *et al.*, 2008). As reported by Onanuga & Temedie (2011), these variations in prevalences of nasal colonization could be linked to the characteristics of study populations. Therefore, the high prevalences of colonization observed on nasal cavities and phones could be attributed to specific characteristics of this study's participants.

5.2 Demographics and colonization and contamination by *S. aureus*

This study established that a more females were colonized by *S. aureus* in their nostrils compared to males **(Table 4-3)**. Possibly, this could be attributed to host behaviors exhibited by

female students within the institution. Although focused on a different study population, this finding agreed with results reported in a study conducted in Madagascar by Hogan *et al.* (2016), where the female gender was significantly associated with nasal carriage of *S. aureus* among health care workers. In other studies, the male gender was found to be significantly associated with nasal colonization by *S. aureus* (Andersen *et al.,* 2013; Neyra *et al.,* 2016). The observation that both significantly high carriages could be observed in both genders could be attributed to the view that host characteristics may play a role in colonization by *S. aureus.* As opposed to contamination on fomites, it was established that the differences in nasal carriage between the six halls of residence were statistically significant. Additionally, nasal colonization was significantly high among students who stayed in groups of four per hostel room. Colonization of nostrils and contamination of phones and pens was found to be high among male students who participated in sports. Also, those who stayed in fours and participated in sports had a significantly high carriage on nostrils, phones and pens. Possibly, this observation could be attribute to the finding participation in sports facilitate physical or close contact, which increases the spread of *S. aureus* (Shen *et al.,* 2013).

5.3 Antimicrobial susceptibility profiles of *S. aureus* and MRSA

Analysis of the antimicrobial susceptibility profiles of the *S. aureus* isolates showed a marked resistance to ampicillin among non-clinical *S. aureus* isolates recovered from a student population in Kenya **(Figure 4-1).** Results reported in this study on ampicillin resistance agreed with those reported in other recent studies like Okamo *et al*. (2016) for isolates recovered from a pre-clinical student population. Kejela & Bacha (2013) reported 100% ampicillin resistance among isolates recovered from healthy a student population. In general, resistances to chloramphenicol, erythromycin, gentamicin and trimethoprim-sulfamethoxazole were low,

ranging between 1.7% and 19.5%. Results in this study indicated that chloramphenicol could be a candidate drug for treatment of staphylococcal infections occurring in this region of central part of Kenya. The present study showed that more than 98% of the isolates were sensitive to chloramphenicol. Compared to MSSA strains, high numbers of MRSA and MDR strains were resistant to erythromycin and trimethoprim-sulfamethoxazole. Gentamicin resistance was low in both strain types. Similar results were reported in Kenya by Aiken *et al.* (2014) but, Maina *et al.* (2013) reported very contrasting results where high resistance was observed among isolates recovered from patients with SSTIs within Nairobi region. It is not clear what factors were behind the difference in resistance levels isolated from patients in the two regions (Thika and Nairobi). Possibly, the origin of the patients could be linked to the resistance difference reported in the two pools of *S. aureus* isolates, but this can be subject to further investigation. Compared to ciprofloxacin, general resistance to norfloxacin was high. In addition, half of isolates that showed resistance to ciprofloxacin were also resistant to drugs in other classes of antibiotics like beta lactams, macrolides, aminoglycosides and even other fluoroquinolones like norfloxacin, hence were classified as multi-drug resistant. As evident in this study, ciprofloxacin cannot be used as an empirical therapy against MRSA-associated infections. Also, *S. aureus* isolates resistant to ciprofloxacin may present a challenge regarding management of infections associated with them since more expensive drugs may be required for therapy.

Resistance to amoxicillin-clavulanic acid was very low, with only a single isolate being resistant to the drug. Additionally, only a single case of intermediate resistance was observed on nitrofurantoin while none of the isolates showed resistance to linezolid. For both MRSA and MSSA strains, their distribution in the susceptible spectrums of amoxicillin-clavulanic acid, nitrofurantoin and linezolid was concentrated around the mid susceptible regions, highlighting

the possibility of the efficacy of these antimicrobial agents being maintained for some time. Therefore, based on the results obtained from this study, amoxicillin clavulanic acid, nitrofurantoin and linezolid could be used against *S. aureus*-related infections with better therapeutic outcomes. It is also crucial not note that inasmuch as amoxicillin clavulanic acid, nitrofurantoin and linezolid may appear as a candidate agents against *S. aureus* in this part of the country, antibiotic susceptibility testing remains paramount.

5.4 Prevalence of MRSA

In the present study, the MRSA strains were detected using cefoxitin (30 μg) discs, which according to Rasheed & Ahmed (2010), is an alternative method to PCR in the detection of MRSA strains. A prevalence of MRSA (11.3%) was found among a healthy population of university students aged between 18-30 years in Central Kenya. The MRSA strains were detected using cefoxitin (30 μg) discs, which according to Rasheed & Ahmed (2010), is an alternative method to PCR in the detection of MRSA strains. So far in Kenya, this is the first study to present results on the prevalence of MRSA among healthy university students residing within institution-based residence halls. The prevalence was found to be high possibly due to the reason the students were not in hospital environments, and they might not have used antibiotics during the time of sample collection. Other recent studies like (Zakai, 2015) have reported a higher MRSA prevalence (18.7%) among student populations in Jeddah, Saudi Arabia while others, Okamo *et al*. (2016) in Tanzania and Kitti *et al.* (2011) in Thailand, have reported very low prevalences of 0.3% and 1% respectively. These reports indicate varied prevalences of MRSA strains in student populations. Even though other reports reveal MRSA prevalences that are as low as 1%, Kitti *et al.* (2011), the prevalences remain higher in other populations in other areas, thus posing a serious public health issue due to frequent contact with colonized individuals

(Zakai, 2015). Based on the results reported by Kitti *et al.* (2011) (an MRSA prevalence of 1% in a student population), it could be possible that non-exposure to MRSA may result in a reduced risks of MRSA colonization. It can be speculated that continued exposure to MRSA colonized environments may result in an increased risk of MRSA colonization. In addition, considering the case of this study where significantly high chances of MRSA colonization were reported in particular residence halls, it could be possible that other students visiting these residence halls would be increasing their chances of MRSA colonization.

5.5 Resistance phenotypes associated with MRSA and MDR strains

This study established that MRSA strains were more likely to display multi-drug resistance compared MSSA strains, a finding which has also been reported by Gupta *et al.* (2013) in India. Among the published studies done in Kenya (all of which focused on clinical isolates), none reported this property among the MRSA strains. Therefore, this study highlights crucial findings regarding the circulation of multi-drug resistant MRSA and MSSA strains among a healthy student population. Given the fact that *S. aureus* is transmitted via direct or indirect person-to-person contact, there is the possibility of an increased prevalence of multi-drug resistant MRSA and MSSA strains among the student population, which is a worrisome scenario. It is also worth noting that the multidrug resistance property renders multi-drug resistant strains as one of the most intractable pathogenic organisms in the history of antibiotic therapy. Therefore, circulation of these multi-drug resistant *S. aureus* among students presents a challenge to management of Staphylococcal infections that may develop, particularly in regards to acquiring effective antibiotics, which may be expensive.

5.6 Genetic diversity of MRSA, MSSA and MDR strains

5.6.1 Prevalence of *mecA* gene among the MRSA strains

Molecular analysis in this study showed that the *S. aureus* isolates were genetically diverse. A majority of the MRSA strains carried the *mecA* gene while none of the MSSA strains carried this gene **(Table 4-6).** Among the studies done in Kenya, carriage of *mecA* gene has been reported among the MRSA strains. In their study, Maina *et al.,* (2013) reported that all the MRSA strains harbored the *mecA* gene, and similar to this study, none of the MSSA strains were found to harbor this gene. In a recent study conducted in Zambia, all the MRSA were found to harbor the *mecA* gene (Samutela *et al.,* 2017). Similar to this study also, a majority of MRSA (90.2%) strains carried *mecA* gene while 9.8% were negative for the gene in a study conducted in Sudan (Elhassan *et al.,* 2015). According to available literature, presence of the *mecA* gene forms the basis of resistance to beta-lactam antibiotics (Murakami *et al.,* 1991; Sharma *et al.,* 1998; Wongwanich *et al.,* 2000). Therefore, basing on suggestions by majority of researchers in this field, the *mecA* gene was linked to cefoxitin resistance among the *mecA*-positive isolates recovered in this study. Other genetic elements may also be considered for the explanation of cefoxitin-resistance mechanisms (Elhassan *et al.,* 2015). Hence, the basis of cefoxitin-resistance among the *mecA*-negative MRSA isolates characterized in this study could be attributed to two possible reasons. Firstly, the hyperproduction of β-lactamases, as described by Olanyika *et al.,* (2009) and secondly, development of specific alterations in the variable amino acids found in the PBPs cascades as highlighted by (Ba *et al.,* 2014).

5.6.2 Prevalence of SCC*mec* elements among MRSA and MDR strains

Screening of all the *S. aureus* isolates for the presence of SCC*mec* elements revealed the existence of SCC*mec*V as the most prevalent *mec* type, followed closely by SCC*mec*II, then SCC*mec*I and lastly, SCC*mec*III, which was only present in three isolates. Contrary to the clinical isolates recovered in Kenya, the non-clinical isolates examined in this study did not harbor any of the SCC*mec*IV types. SCC*mec*V has been shown to be of a small molecular size, and this could be the possible reason for its high prevalence among isolates recovered in this study (Omuse *et al.,* 2016). No other published study done in Kenya with similar findings was found. Previously, SCC*mec*IV strains have been reported to circulate highly in community settings, possibly because of its small size, which renders it more mobile and thus can be inserted into multiple *S. aureus* lineages (Omuse *et al.,* 2016; Tong *et al.,* 2012). It was unclear why none of the isolates recovered in this study harbored SCC*mec*IV.

The prevalence of SCC*mec*I was high among the MRSA strains compared to MDR strains. This observation was not unique to this study since SCC*mec*I is known to encode solely for resistance to β-lactam antimicrobials (Deurenberg *et al.,* 2007). The prevalence of SCC*mec*II was found to be high among the MDRs compared to MRSA strains. Also, this observation was not unique to the present study because the SCC*mec*II cassettes are determinants of antimicrobial multiresistance. The SCC*mec*II cassette carries additional genes for drug resistance, which are integrated in plasmids (pI258, pUB110 and pT181) and a transposon (Tn554) (Deurenberg *et al.,* 2007). Similar to SCC*mec*II, the prevalence of SCC*mec*V was found to be slightly higher among the MDRs than MRSAs by 1%. SCC*mec*II (as stated before) determines multiresistance while SCC*mec*V encodes for resistance to β-lactam antimicrobial. The high numbers of SCC*mec*V

strains, which were also found to be MDRs could be attributed possibly to the fact that half the population of MDRs carried both SCC*mec*II and V in their genomes.

5.6.3 Prevalence of multiple SCC*mec* elements

In this study, carriage of multiple SCC*mec* elements was reported on several occasions. A similar observation was made by Maina *et al.,* (2013) in Kenya where a single isolate was found to carry both SCC*mec* I and II. Almost half of the MRSA strains analyzed in the present study carried SCC*mec*II and SCC*mec*V compared to those that carried SCC*mec*I and SCC*mec*V (11.5%), SCC*mec*I and SCC*mec*III (7.7%) and SCC*mec*II and SCC*mec*V (3.9%). Half the proportion of MDRs carried SCC*mec*II and SCC*mec*V. Characteristically, this study established that the SCC*mec*II and SCC*mec*V combination was more prevalent among the MRSA and MDR strains, and therefore, a likely genotypic characteristic of MRSA strains that are also MDRs. More studies could also be done to ascertain the prevalence of the existence of this combination of SCC*mec* elements among MRSA strains that are also MDRs.

5.6.4 Variants of SCC*mec* elements

In the present study, two MSSA strains were also found to carry SCC*mec*I. Additionally, among the *mecA*-negative MRSA strains, six tested positive for SCC*mec*II, five tested positive for SCC*mec*V while only a single isolate tested positive for SCC*mec*III. None of the previously published studies done in Kenya reported a similar finding. This finding was unclear, though other research reports demonstrated the existence of similar observations (Chlebowicz *et al.,* 2010; Donnio *et al.,* 2005; International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009; Shore *et al.,* 2014; Vandendriessche *et al.,* 2014; Wong, Louie, Lo, & Simor, 2010). Donnio *et al.* (2005) characterized MSSA strains,

which tested positive for the IS431: pUB110: IS431::DCS structure (SCC*mec*-associated elements), but negative for *mecA* and *ccrAB.* Wong *et al.* (2010) characterized seven isolates, which resembled USA100 and tested positive for SCC*mec*II but negative for *mecA.* For the two MSSA isolates that carried SCC*mec*I, it could be possible that the isolates lost the methicillin-resistant phenotype due to partial excision of the SCC*mec* Stamper *et al.* (2011). On the other hand, the *mecA*-negative MRSA carrying the various SCC*mec* types retained their methicillin-resistant phenotype possibly due to other genetic factors, which have been described previously in this context.

5.6.5 Prevalence of *lukFS-PV* gene

Slightly more than a quarter of the sampled isolates testing positive for the *lukFS-PV* gene. The study also showed that a large proportion of the MSSA carried the *lukFS-PV* gene, compared to the proportion of MRSAs and MDRs. The difference in carriage of this gene between the MSSA and MRSA strains was statistically insignificant (p value = 0.487). In terms of overall prevalence of *lukFS-PV* gene among *S. aureus* isolates recovered in Kenya, these findings were not unique to this study. In previous study done in Kenya on non-clinical isolates, the overall carriage of *lukFS-PV* gene (20%) was lower than the overall prevalence reported in the present study. In another study done by Maina et al. (2013), a slightly lower proportion of *lukFS-PV* positive MRSA strains (20.3%) than this study's was found. Aiken et al. (2014) reported an overall *lukFS-PV* carriage of 19%. In this same study, none of the MRSA strains (isolated from mostly burn patients) carried this gene, a finding that could possibly agree with the idea that PVL is commonly associated with CA-MRSA (Vandenesch et al., 2003). In a study that focused on an African population, a pandemic clone (ST 152), which comprised of 23.9% of carriage isolates was identified and all isolates in this clone were PVL strains (Ruimy *et al.*, 2008). Basing on this

finding, and also as speculated by Omuse *et al.,* (2013), it could be possible that there is a *S. aureus* clone carrying the *lukFS-PV* gene circulating among the student population. Presently, the significance of the high carriage of the *lukFS-PV* among *S. aureus* isolates colonizing the student population remains unclear, and as such, warrants continued investigations that may aid in the determination of the disease causing potential of the respective *S. aureus* strains.

5.6.6 Genetic relatedness of *S. aureus* isolates recovered from the student population

In the present study, the REP-PCR was used successfully to assess the phylogenetic relationships among the *S. aureus* isolates. The respective analysis of genetic relations of the isolates indicated a likelihood of strain sharing among students **(Figure 4-14)**. This would be expected since the isolates were recovered from students who stayed in close proximities, and shared their personal items. The isolates appeared to cluster based on the gender of the participants from which the isolate was obtained. All isolates in cluster A, except one, were isolated from female participants. Since students of the same gender lived in one hostel block, it could be possible that that isolates in the respective cluster circulated within the female residence halls. These isolates were from different female hostels. Possibly, this indicated that the students from where the isolates were recovered had other contacts from other female halls, a factor which facilitated strain sharing between students from different female residence halls. The single isolate recovered from a male student could also have been picked by the male student from a female contact within the female residence halls. It was not clear why isolates from male's residence halls did not show signs of strain sharing. However, it was thought that these isolates could have been picked from other contacts outside the residence halls. To cement this observation, isolates belonging to the other clusters (which were a mixture of those isolated from male and female students) appeared to

show some degree of similarity on the basis of being positive or negative for the *mecA* gene. Based on the evidence of strain sharing noted in female residence halls, the mode of occupancy observed in all female residence halls could be implicated in increasing the likelihood of colonization by *S. aureus*, especially in the nose. Since, phylogenetic analysis showed evidence of strain sharing particularly in this halls, it can be speculated that indeed, this high number of students per room facilitated increased rates of bacterial transmission.

5.7 Possible risk factors for colonization by *S. aureus*, MRSA and MDR strains

Based on the findings reported in this study, that the female gender was a possible risk factor for colonization by *S. aureus* in the nasal cavity. The fact that either gender has been reported to be a risk factor for nasal colonization by *S. aureus* may cement the view that variations in prevalences of nostril colonization could be linked to the characteristics of respective population groups. Staying in Halls E, C and B was found to be a possible risk factor for nasal colonization by MRSA while staying in groups of four per hostel room was a possible risk factor for nasal colonization by *S. aureus*. This was a typical indication that staying four students per hostel room resembled congested or crowded living conditions. Crowded living conditions have been identified as risk factors for colonization by *S. aureus* on a number of occasions (Eibach *et al.,* 2017; Geraci *et al.,* 2014; Mukherjee *et al.,* 2008; Pathak *et al.,* 2010). Different studies examining the transmission of infectious diseases have found out that the degree of contact between infected or colonized individuals and other group members plays a fundamental role in both disease incidences and prevalences in any setting (Miller *et al.,* 2009; Price *et al.,* 2017). This study therefore, reveals the negative impact associated with certain of occupancy implemented within the institution, thus prompting necessary steps to be undertaken to help reduce the number of students allocated in one hostel room. Participation in sports was also

established as a possible risk factor for colonization by *S. aureus* on nostrils, phones and pens of male students. Additionally, the study showed that those who participated in sports and stayed in high numbers (four students per room) were at a high risk of colonization on their nostrils and contamination of phones and pens. Of importance to note also, is that, *S. aureus* related outbreaks have been reported among students who participated in college sports (Fontanilla *et al.,* 2010; Nguyen *et al.,* 2005; Romano *et al.,* 2006). These reports highlight the risk of colonization that is associated with index persons carrying the infectious strains of *S. aureus* making contact with other team mates. These findings are crucial since they confirm that participation in sports increases the likelihood of colonization by *S. aureus*, and also reveal the extent of risk associated with staying in crowded conditions particularly for those who are already at risk by taking part in sports. Failure to disinfect phones was established as possible risk factors for colonization by MRSA strains. These findings highlight the possible advantages of ensuring proper hygiene by disinfecting phones and pens to limit cases of MRSA transmission through direct and indirect contact. The study also established that sharing of personal effects could be a probable risk factor for colonization by MDR strains among the students. No studies with similar findings in Kenya were found and therefore, this study reveals crucial information regarding transmission of MRSA, and requires more studies to be done to provide more information regarding these transmission dynamics.

CHAPTR SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. The study demonstrated existence of a high prevalence of *S. aureus* and MRSA among the student population. The respective prevalences of *S. aureus* and MRSA remain to be of epidemiological significance since the circulating strains may be transmitted to other non-colonized individuals, thus increasing the prevalence and also contributing to the likelihood of an MRSA outbreak lest preventive measures are observed.
2. The present study demonstrated the possibility that amoxicillin-clavulanic acid, nitrofurantoin and linezolid could be used effectively in the management of *S. aureus* related infections. Nonetheless, due to the current trends in development of antimicrobial resistance, performing antimicrobial susceptibility tests prior to administration is still crucial.
3. The study demonstrated that MRSA strains have a high likelihood of displaying multidrug resistance. This property among the MRSA strains presents a worrisome scenario in regards to management of Staphylococcal infections that may be caused by MRSA strains. Possibly, drugs that may be effective against these strains may be expensive to purchase, particularly in resource-limited countries. Therefore, observing preventive measure to curb transmission remains paramount.
4. Analysis of genetic relations of the isolates indicated a likelihood of strain sharing among students. It is therefore crucial to observe these factors while implementing strategies to curb spread of *S. aureus* of MRSA among students.

5. The study demonstrated that being female, staying in groups of four per hostel room, participating in sports, failure to disinfect phones, and using non-medicated soaps were factors for nasal colonization, and phone or pen contamination by either *S. aureus* or MRSA.

6.2 Recommendations

1. To lower the prevalence of *S. aureus* and MRSA within the University student population, the number of students sharing a hostel room should be limited to less than four students per room to avoid crowded living conditions. Students participating in sports should clean their hands at least before and after taking part in sports. All students should adopt behaviors of disinfecting their phones and pens and washing hands using medicated soaps.
2. Treatment of Staphylococcal infections should only be guided by culture and antimicrobial susceptibility profiles and available treatment regimes. This is particularly crucial since not all antimicrobials are the MSSA, MRSA and MDR strains tend to behave differently on different antimicrobials. Treatment with amoxicillin-clavulanic acid, nitrofurantoin and linezolid can be considered especially due to their better therapeutic results against the MRSA and MDR strains.
3. This study focused on a small population of students in Kenya, and therefore, conclusions on factors linked to colonization and contamination by *S. aureus* or MRSA may not be drawn based on results from this study alone. Similar studies should be carried through the country out to identify factors contributing to spread of *S. aureus* and MRSA, particularly within university settings.

REFERENCES

Abad, C., Fearday, A., & Safdar, N. (2010). Adverse effects of isolation in hospitalised patients: a systematic review. *The Journal of Hospital Infection*, *76*(2), 97–102.

Aiken, A. M., Mutuku, I. M., Sabat, A. J., Akkerboom, V., Mwangi, J., Scott, J. A. G., … Grundmann, H. (2014). Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. *Antimicrobial Resistance and Infection Control*, *3*, 1–7.

Andersen, P. S., Larsen, L. A., Fowler, V. G., Stegger, M., Skov, R. L., & Christensen, K. (2013). Risk factors for *Staphylococcus aureus* nasal colonization in Danish middle-aged and elderly twins. *European Journal of Clinical Microbiology and Infectious Diseases*, *32*(10), 1321–1326.

Ansari, S., Gautam, R., Shrestha, S., Ansari, S. R., Subedi, S. N., & Chhetri, M. R. (2016). Risk factors assessment for nasal colonization of *Staphylococcus aureus* and its methicillin resistant strains among pre-clinical medical students of Nepal. *BMC Research Notes*, *9*(1), 1–8.

Arêde, P., Milheiriço, C., de Lencastre, H., & Oliveira, D. C. (2012). The anti-repressor MecR2 promotes the proteolysis of the mecA repressor and enables optimal expression of β-lactam resistance in MRSA. *PLoS Pathogens*, *8*(7), 1–17.

Arulmozhi, V., Janagond, A. B., Savitha, S., Kalyani, J., & Sumathi, G. (2014). Cell phones of healthcare providers as fomites harbouring potential pathogenic microogranisms. *International Journal of Current Research and Review*, *6*(19), 1–4.

Ba, X., Harrison, E. M., Edwards, G. F., Holden, M. T. G., Larsen, A. R., Petersen, A., … Holmes, M. A. (2014). Novel mutations in penicillin-binding protein genes in clinical *Staphylococcus aureus* isolates that are methicillin resistant on susceptibility testing, but lack the mec gene. *Journal of Antimicrobial Chemotherapy*, *69*(3), 594–597.

Bannerman, T. L., Hancock, G. A., Tenover, F. C., & Miller, J. M. (1995). Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *33*(3), 551–555.

Banniettis, N., Beekmann, S. E., Polgreen, P. M., Kaushik, S., Kohlhoff, S., Gilbert, D., … Hammerschlag, M. R. (2018). Management Practices for Methicillin-Resistant *Staphylococcus aureus* Bacteremia by Adult Infectious Diseases Physicians. *Open Forum Infectious Diseases*, *5*(5).

Baron, S. (1996). *Medical Microbiology*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21413252

Ben Slama, K., Gharsa, H., Klibi, N., Jouini, A., Lozano, C., G?mez-Sanz, E., … Torres, C. (2011). Nasal carriage of *Staphylococcus aureus* in healthy humans with different levels of contact with animals in Tunisia: genetic lineages, methicillin resistance, and virulence factors. *European Journal of Clinical Microbiology & Infectious Diseases*, *30*(4), 499–508.

Brown, A. F., Leech, J. M., Rogers, T. R., & McLoughlin, R. M. (2014). *Staphylococcus aureus* colonization: Modulation of host immune response and impact on human vaccine design. *Frontiers in Immunology*, *4*(507), 1–20.

Calfee, D. P., Salgado, C. D., Milstone, A. M., Harris, A. D., Kuhar, D. T., Moody, J., … Yokoe,

D. S. (2014). Strategies to Prevent Methicillin-Resistant *Staphylococcus aureus* Transmission and Infection in Acute Care Hospitals: 2014 Update. *Infection Control and Hospital Epidemiology*, *35*(7), 772–796.

Centers for Disease Control and Prevention (CDC). (2002). Vancomycin-resistant *Staphylococcus aureus*--Pennsylvania, 2002. *MMWR. Morbidity and Mortality Weekly Report*, *51*(40), 902.

Centers for Disease Control and Prevention (CDC). (2013). *Antibiotic Resistance Threats in the United States*. *CDC*.

Chambers, H. F., & Deleo, F. R. (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Reviews Microbiology*, *7*(9), 629–641.

Champion, A. E., Goodwin, T. A., Brolinson, P. G., Werre, S. R., Prater, M. R., & Inzana, T. J. (2014). Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from healthy university student athletes. *Annals of Clinical Microbiology and Antimicrobials*, *13*(1), 33.

Chang, S., Sethi, A. K., Stiefel, U., Cadnum, J. L., & Donskey, C. J. (2010). Occurrence of Skin and Environmental Contamination with Methicillin-Resistant *Staphylococcus aureus* before Results of Polymerase Chain Reaction at Hospital Admission Become Available. *Infection Control & Hospital Epidemiology*, *31*(06), 607–612.

Charlebois, E. D., Perdreau-Remington, F., Kreiswirth, B., Bangsberg, D. R., Ciccarone, D., Diep, B. A., … Chambers, H. F. (2004). Origins of community strains of methicillin-resistant *Staphylococcus aureus*. *Clinical Infectious Diseases : An Official Publication of*

the Infectious Diseases Society of America, *39*(1), 47–54.

Chatterjee, S. S., & Otto, M. (2013). Improved understanding of factors driving epidemic waves. *Clinical Epidemiology*, *5*, 205–217.

Chen, C. S., Chen, C. Y., & Huang, Y. C. (2012). Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university. *International Journal of Infectious Diseases*, *16*(11), e789–e793.

Chlebowicz, M. A., Nganou, K., Kozytska, S., Arends, J. P., Engelmann, S., Grundmann, H., … Buist, G. (2010). Recombination between ccrC genes in a type V (5C2&5) staphylococcal cassette chromosome mec (SCCmec) of *Staphylococcus aureus* ST398 leads to conversion from methicillin resistance to methicillin susceptibility in vivo. *Antimicrobial Agents and Chemotherapy*, *54*(2), 783–791.

CLSI. (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25. *Clinical and Laboratory Standards Institute*, (January).

Cochran, W. G. (1963). *Sampling techniques.* (2nd edition). New York: John Wiley & Sons.

Coia, J. E., Duckworth, G. J., Edwards, D. I., Farrington, M., Fry, C., Humphreys, H., … Tucker, D. R. (2006). Guidelines for the control and prevention of meticillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities. *Journal of Hospital Infection*, *63*(SUPPL. 1), 1–44.

Creech, C. B., Kernodle, D. S., Alsentzer, A., Wilson, C., & Edwards, K. M. (2005). Increasing

rates of nasal carriage of methicillin-resistant *Staphylococcus aureus* in healthy children. *The Pediatric Infectious Disease Journal*, *24*(7), 617–621.

David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews*, *23*(3), 616–687.

Deplano, A., Vaneechoutte, M., Verschraegen, G., & Struelens, M. J. (1997). Typing of *Staphylococcus aureus* and Staphylococcus epidermidis strains by PCR analysis of inter-IS256 spacer length polymorphisms. *Journal of Clinical Microbiology*, *35*(10), 2580–2587.

Deresinski, S. (2007). Counterpoint: Vancomycin and *Staphylococcus aureus*--An Antibiotic Enters Obsolescence. *Clinical Infectious Diseases*, *44*(12), 1543–1548.

Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A., & Stobberingh, E. E. (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection*, *13*(3), 222–235.

Diederen, B. M. W., Kluytmans, J. A. J. W., Butterworth, L. A., Hopley, A. L., Nicholson, A., Gould, F. K., & al., et. (2006). The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus*. *The Journal of Infection*, *52*(3), 157–168.

Donnio, P., Oliveira, D. C., Faria, N. A., Wilhelm, N., Coustumier, A. Le, De, H., & Lencastre, H. De. (2005). Partial Excision of the Chromosomal Cassette Containing the Methicillin Resistance Determinant Results in Methicillin-Susceptible *Staphylococcus aureus* Partial Excision of the Chromosomal Cassette Containing the Methicillin Resistance Determinant Results. *Journal of Clinical Microbiology*, *43*(8), 4191–4193.

Du, J., Chen, C., Ding, B., Tu, J., Qin, Z., Parsons, C., … Yu, F. (2011). Molecular characterization and antimicrobial susceptibility of nasal *staphylococcus aureus* isolates from a chinese medical college campus. *PLoS ONE*, *6*(11), 1–5.

Eibach, D., Nagel, M., Hogan, B., Azuure, C., Krumkamp, R., Dekker, D., … May, J. (2017). Nasal carriage of *staphylococcus aureus* among children in the Ashanti region of Ghana. *PLoS ONE*, *12*(1), 1–11.

Elhassan, M. M., Ozbak, H. A., Hemeg, H. A., Elmekki, M. A., & Ahmed, L. M. (2015). Absence of the *mec* A Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Specimens in Shendi City, Sudan. *BioMed Research International*, *2015*, 1–5.

Ellington, M. J., Ganner, M., Warner, M., Cookson, B. D., & Kearns, A. M. (2010). Polyclonal multiply antibiotic-resistant methicillin-resistant *Staphylococcus aureus* with Panton – Valentine leucocidin in England. *J Antimicrob Chemother*, *65*, 46–50.

Ellington, M. J., Perry, C., Ganner, M., Warner, M., McCormick Smith, I., Hill, R. L., … Kearns, A. M. (2009). Clinical and molecular epidemiology of ciprofloxacin-susceptible MRSA encoding PVL in England and Wales. *European Journal of Clinical Microbiology & Infectious Diseases*, *28*(9), 1113–1121.

Farley, J. E., Ross, T., Stamper, P., Baucom, S., Larson, E., & Carroll, K. C. (2008). Prevalence, risk factors, and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among newly arrested men in Baltimore, Maryland. *American Journal of Infection Control*, *36*(9), 644–650.

Finks, J., Wells, E., Dyke, T. L., Husain, N., Plizga, L., Heddurshetti, R., … Miller, C. (2009). Vancomycin-resistant *Staphylococcus aureus*, Michigan, USA, 2007. *Emerging Infectious Diseases*, *15*(6), 943–945.

Fontanilla, J. M., Kirkland, K. B., Talbot, E. A., Powell, K. E., Schwartzman, J. D., Goering, R. V., & Parsonnet, J. (2010). Outbreak of skin infections in college football team members due to an unusual strain of community-acquired methicillin-susceptible *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *48*(2), 609–611.

Fuda, C., Suvorov, M., Vakulenko, S. B., & Mobashery, S. (2004). The basis for resistance to β-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. *Journal of Biological Chemistry*, *279*(39), 40802–40806.

Gardete, S., & Tomasz, A. (2014). Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *The Journal of Clinical Investigation*, *124*(7), 2836–2840.

Geraci, D. M., Giuffrre, M., Bonura, C., Matranga, D., Aleo, A., Saporito, L., … Mammina, C. (2014). Methicillin-resistant *Staphylococcus aureus* colonization: A three-year prospective study in a neonatal intensive care unit in Italy. *PLoS ONE*, *9*(2), 1–9.

Goering, R. V., Larsen, A. R., Skov, R., Tenover, F. C., Anderson, K. L., & M., P. D. (2009). Comparative genomic analysis of European and Middle Eastern community-associated methicillin-resistant *Staphylococcus aureus* (CC80:ST80-IV) isolates by high-density microarray. *Clin Microbiol Infect.*, *15*(8), 748–755.

Goudarzi, M., Goudarzi, H., Sá Figueiredo, A. M., Udo, E. E., Fazeli, M., Asadzadeh, M., & Seyedjavadi, S. S. (2016). Molecular Characterization of Methicillin Resistant

Staphylococcus aureus Strains Isolated from Intensive Care Units in Iran: ST22-SCCmec IV/t790 Emerges as the Major Clone. *PLOS ONE*, *11*(5), e0155529.

Gupta, R., Nanda, S., & Barve, S. (2013). Multi drug resistance in methicillin-resistant *staphylococcus aureus* (MRSA) isolates from a university hospital of western India. *Journal of Evolution of Medical and Dental Sciences*, *2*(9), 1354–1358.

Hamdan-Partida, A., Sainz-Espuñes, T., & Bustos-Martínez, J. (2010). Characterization and persistence of *Staphylococcus aureus* strains isolated from the anterior nares and throats of healthy carriers in a Mexican community. *Journal of Clinical Microbiology*, *48*(5), 1701–1705.

Hassoun, A., Linden, P. K., & Friedman, B. (2017). Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment. *Critical Care*, *21*(1), 211.

Hidron, A. I., Kourbatova, E. V., Halvosa, J. S., Terrell, B. J., McDougal, L. K., Tenover, F. C., … King, M. D. (2005). Risk Factors for Colonization with Methicillin?Resistant *Staphylococcus aureus* (MRSA) in Patients Admitted to an Urban Hospital: Emergence of Community?Associated MRSA Nasal Carriage. *Clinical Infectious Diseases*, *41*(2), 159–166.

Hiramatsu, K. (1998). The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. *The American Journal of Medicine*, *104*(5A), 7S–10S.

Hiramatsu, K., Aritaka, N., Hanaki, H., Kawasaki, S., Hosoda, Y., Hori, S., … Kobayashi, I. (1997). Dissemination in Japanese hospitals of strains of *Staphylococcus aureus*

heterogeneously resistant to vancomycin. *Lancet (London, England)*, *350*(9092), 1670–1673.

Hogan, B., Rakotozandrindrainy, R., Al-emran, H., Dekker, D., Hahn, A., Jaeger, A., … Schwarz, N. G. (2016). Prevalence of nasal colonisation by resistant *Staphylococcus aureus* among healthcare workers and students in Madagascar. *BMC Infectious Diseases*, 1–9.

Holland, T. L., Arnold, C., Fowler, V. G., & Jr. (2014). Clinical management of *Staphylococcus aureus* bacteremia: a review. *JAMA*, *312*(13), 1330–1341.

Holmes, A., Ganner, M., Mcguane, S., Pitt, T. L., Cookson, B. D., & Kearns, A. M. (2005). *Staphylococcus aureus* Isolates Carrying Panton-Valentine Leucocidin Genes in England and Wales : Frequency , Characterization , and Association with Clinical Disease. *Journal of Clinical Microbiology*, *43*(5), 2384–2390.

Holubar, M., Meng, L., & Deresinski, S. (2016). Bacteremia due to Methicillin-Resistant *Staphylococcus aureus*: New Therapeutic Approaches. *Infectious Disease Clinics of North America*, *30*(2), 491–507.

Huijsdens, X. W., Lier, A. M. C., Kregten, E. Van, Verhoef, L., Santen-verheuvel, Van, M. G., … Wannet, W. J. B. (2006). Methicillinresistant *Staphylococcus aureus* in Dutch Soccer Team. *Emerg Infect Dis*, *12*(10), 1584–1586.

HwangBo, K., Son, S. H., Lee, S. J., Min, R. S., Ko, M. S., Liu, R. J., … Jeong, J. W. (2010). Rapid and simple method for DNA extraction from plant and algal species suitable for PCR amplification using a chelatin resin Chelex 100. *Plant Biotechnol Rep*, (4), 49–52.

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), I. W. G. on the C. of S. C. C. E. (2009). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrobial Agents and Chemotherapy*, *53*(12), 4961–4967.

Jevons, M. P. (1961). "Celbenin"- Resistant Staphylococci. *British Medical Journal*, *1*, 124–125.

Jiménez-Truque, N., Saye, E. J., Soper, N., Saville, B. R., Thomsen, I., Edwards, K. M., & Creech, C. B. (2017). Association Between Contact Sports and Colonization with *Staphylococcus aureus* in a Prospective Cohort of Collegiate Athletes. *Sports Medicine (Auckland, N.Z.)*, *47*(5), 1011–1019.

Jung, S.-O., Moon, Y. M., Kim, S.-H., Sung, H. Y., Kwon, S.-J., Kang, Y. H., & Yu, J. Y. (2011). Multilocus Sequence Analysis of Housekeeping Genes and Antigenic Determinant Genes in Bordetella pertussis Strains Isolated in Korea. *Osong Public Health and Research Perspectives*, *2*(2), 115–126.

Katayama, Y., Ito, T., & Hiramatsu, K. (2000). A New Class of Genetic Element , Staphylococcus Cassette Chromosome mec , Encodes Methicillin Resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *44*(6), 1549–1555.

Katiyar, R., Deorukhkar, S. C., & Saini, S. (2011). What does a pen carry? Are you aware? *International Journal of Biomedical Research*, *6*, 346–352.

Kejela, T., & Bacha, K. (2013). Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. *Annals of Clinical Microbiology and Antimicrobials*,

12(1), 11.

Kitti, T., Boonyonying, K., & Sitthisak, S. (2011). Prevalence of methicillin-resistant *Staphylococcus aureus* among university students in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *42*(6), 1498–1504.

Kollef, M. H. (2007). Limitations of Vancomycin in the Management of Resistant Staphylococcal Infections. *Clinical Infectious Diseases*, *45*(Supplement_3), S191–S195.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J., & Hiramatsu, K. (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: Rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrobial Agents and Chemotherapy*, *51*(1), 264–274.

Kullar, R., Casapao, A. M., Davis, S. L., Levine, D. P., Zhao, J. J., Crank, C. W., … Rybak, M. J. (2013). A multicentre evaluation of the effectiveness and safety of high-dose daptomycin for the treatment of infective endocarditis. *The Journal of Antimicrobial Chemotherapy*, *68*(12), 2921–2926.

Kumari, D. N., Keer, V., Hawkey, P. M., Parnell, P., Joseph, N., Richardson, J. F., & Cookson, B. (1997). Comparison and application of ribosome spacer DNA amplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. *Journal of Clinical Microbiology*, *35*(4), 881–885.

L. G. Miller, & Diep, B. A. (2008). Colonization, Fomites, and Virulence: Rethinking the Pathogenesis of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Infection. *Clinical Infectious Diseases*, *46*(5), 752–760.

Lear, A., Mccord, G., & Peiffer, J. (2011). Incidence of *Staphylococcus aureus* Nasal Colonization and Soft Tissue Infection Among High School Football Players. *The Journal of the American Board of Family Medicine*, *44307*, 429–435.

Lina, G., Piémont, Y., Godail-Gamot, F., Bes, M., Peter, M.-O., Gauduchon, V., … Etienne, J. (1999). Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *29*(5), 1128–1132.

Logman, J. F. S., Stephens, J., Heeg, B., Haider, S., Cappelleri, J., Nathwani, D., … van Hout, B. A. (2010). Comparative effectiveness of antibiotics for the treatment of MRSA complicated skin and soft tissue infections. *Current Medical Research and Opinion*, *26*(7), 1565–1578.

Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., … Spratt, B. G. (1998). Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(6), 3140–3145. Retrieved from

Maina, E. K., Kiiyukia, C., Wamae, C. N., Waiyaki, P. G., & Kariuki, S. (2013). Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya. *International Journal of Infectious Diseases*, *17*(2), e115–e119.

Marimuthu, K., Pittet, D., & Harbarth, S. (2014). The effect of improved hand hygiene on nosocomial MRSA control. *Antimicrobial Resistance and Infection Control*, *3*, 34.

Mbogori, C., Muigai, A., & Kariuki, S. (2013). Detection and Characterization of Methicillin

Resistant *Staphylococcus aureus* from Toilet and Classroom Door Handles in Selected Secondary Schools in Nairobi County. *Open Journal of Medical Microbiology*, *3*, 248–252.

Melles, Damian C; van Leeuwen, Willem B; Boelens, Helene AM;Peeters, Justine K;Verbrugh, H. a; V. B. (2006). Panton-Valentine Leukocidin Genes in Staphylococcus Small Anellovirus in Hepatitis C Patients and Healthy Controls. *Emerging Infectious Diseases*, *12*(7), 1174–1175.

Melles, D. C., Gorkink, R. F. J., Boelens, H. A. M., Snijders, S. V, Peeters, J. K., Moorhouse, M. J., … Belkum, A. Van. (2004). Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. *The Journal of Clinical Investigation*, *114*(12), 1732–1740.

Melzer, M., & Welch, C. (2013). Outcomes in UK patients with hospital-acquired bacteraemia and the risk of catheter-associated urinary tract infections. *Postgrad Med J.*, *89*, 329–334.

Miller, L. G., Eells, S. J., Taylor, A. R., David, M. Z., Ortiz, N., Zychowski, D., … Daum, R. S. (2012). *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *54*(11), 1523–1535.

Miller, M., Cook, H. A., Furuya, E. Y., Bhat, M., Lee, M. H., Vavagiakis, P., … Lowy, F. D. (2009). *Staphylococcus aureus* in the community: Colonization versus infection. *PLoS ONE*, *4*(8), 1–9.

Moise, P. A., Amodio-Groton, M., Rashid, M., Lamp, K. C., Hoffman-Roberts, H. L., Sakoulas,

G., … Rastogi, A. (2013). Multicenter evaluation of the clinical outcomes of daptomycin with and without concomitant β-lactams in patients with *Staphylococcus aureus* bacteremia and mild to moderate renal impairment. *Antimicrobial Agents and Chemotherapy*, *57*(3), 1192–1200.

Morgan, D. J., Rogawski, E., Thom, K. A., Johnson, J. K., Perencevich, E. N., Shardell, M., … Harris, A. D. (2012). Transfer of multidrug-resistant bacteria to healthcare workers' gloves and gowns after patient contact increases with environmental contamination. *Critical Care Medicine*, *40*(4), 1045–1051.

Mozzillo, K. L., Ortiz, N., & Miller, L. G. (2010). NIH Public Access. *J Hosp Infect*, *75*(2), 132–134.

Mukherjee, D. V., Herzig, C. T. A., Jeon, C. Y., Lee, C. J., Apa, Z. L., Genovese, M., … Larson, E. L. (2008). Prevalence and risk factors for *Staphylococcus aureus* colonization in individuals entering maximum-security prisons. *Epidemiol Infect*, *6*(9), 2166–2171.

Mulcahy, M. E., Geoghegan, J. A., Monk, I. R., O'Keeffe, K. M., Walsh, E. J., Foster, T. J., & McLoughlin, R. M. (2012). Nasal Colonisation by *Staphylococcus aureus* Depends upon Clumping Factor B Binding to the Squamous Epithelial Cell Envelope Protein Loricrin. *PLoS Pathogens*, *8*(12), 1–14.

Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H., & Watanabe, S. (1991). Identification of methicillin-resistant staphylococci by multiplex polymerase chain reaction assay. *Am.J.Clin.Pathol.*, *29*(10), 2240–2244.

Muthotho, J. N., Waiyaki, P. G., Mbalu, M., Wairugu, A., Mwanthi, B., & Odongo, B. (1995).

Control of spread of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Burns Units. *African Journal of Health Sciences*, *2*(1), 232–235.

Navratna, V., Nadig, S., Sood, V., Prasad, K., Arakere, G., & Gopal, B. (2010). Molecular Basis for the Role of *Staphylococcus aureus* Penicillin Binding Protein 4 in Antimicrobial Resistance. *Journal of Bacteriology*, *192*(1), 134–144.

Neyra, J., Ellis, M., Rocha, C., Silvera, J., Apolaya, M., Bernal, M., … Andrade, A. (2016). Prevalence of Nasal colonization with *Staphylococcus aureus* in 4 cities in Peru. *Tropical Diseases, Travel Medicine and Vaccines*, *2*(1), 12.

Nguyen, D. M., Mascola, L., & Bancroft, E. (2005). Recurring methicillin-resistant *Staphylococcus aureus* infections in a football team. *Emerging Infectious Diseases*, *11*(4), 526–532.

Okamo, B., Moremi, N., Seni, J., Mirambo, M. M., Kidenya, B. R., & Mshana, S. E. (2016). Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* nasal carriage among pre-clinical and clinical medical students in a Tanzanian University. *BMC Research Notes*, *9*(1), 47.

Olanyika, B. ., Olanyika, A. ., Obajuluwa, A. ., Onaolapo, J. ., & Olurinola, P. . (2009). Absence of mecA gene in methicillin-resistant *Staphylococcus aureus* isolates. *Afr. J. Infect. Dis.*, *5*(4), 383–389.

Omuse, G., Shivachi, P., Kariuki, S., & Revathi, G. (2013). Prevalence of Panton Valentine Leukocidin in Carriage and Infective Strains of *Staphylococcus aureus* at a Referral Hospital in Kenya. *Open Journal of Medical Microbiology*, *3*, 5–11.

Omuse, G., Van Zyl, K. N., Hoek, K., Abdulgader, S., Kariuki, S., Whitelaw, A., & Revathi, G. (2016). Molecular characterization of *Staphylococcus aureus* isolates from various healthcare institutions in Nairobi, Kenya: a cross sectional study. *Annals of Clinical Microbiology and Antimicrobials*, *15*(1), 51.

Onanuga, A., & Temedie, T. C. (2011). Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger delta region of Nigeria. *African Health Sciences*, *11*(2), 176–181.

Pathak, A., Marothi, Y., Iyer, R. V, Singh, B., Sharma, M., Eriksson, B., … Lundborg, C. S. (2010). Nasal Carriage and Antimicrobial Susceptibility of *Staphylococcus aureus*in healthy preschool children in Ujjain, India. *BMC Pediatrics*, *10*(1), 100.

Pires, F. V., Da Cunha, M. D. L. R. D. S., Abraão, L. M., Martins, P. Y. F., Camargo, C. H., & Fortaleza, C. M. C. B. (2014). Nasal carriage of *Staphylococcus aureus* in Botucatu, Brazil: A population-based survey. *PLoS ONE*, *9*(3), 1–7.

Power, E. G. (1996). RAPD typing in microbiology--a technical review. *The Journal of Hospital Infection*, *34*(4), 247–265.

Price, J. R., Cole, K., Bexley, A., Kostiou, V., Eyre, D. W., Golubchik, T., … Paul, J. (2017). Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *The Lancet Infectious Diseases*, *17*(2), 207–214.

Qiao, Y., Xue, N., Qiang, C., Zhao, R., Song, W., Zheng, Y., … Wang, L. (2014). Clinical and molecular characteristics of invasive community-acquired *Staphylococcus aureus* infections

in Chinese children. *Bmc Infectious Diseases*, *14*(1), 582.

Rasheed, M. U., & Ahmed, Z. (2010). Phenotypic methods of greater accuracy to detect the mecA gene product for the recognition of MRSA in resource constraint settings. *Asian Pacific Journal of Tropical Medicine*, *3*(9), 741–744.

Rijal, K. R., Pahari, N., Shrestha, B. K., Paudel, B., & Mahato, P. (2008). Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara. *Nepal Med Coll J*, *10*(3), 192–195.

Rodriguez, M., Hogan, P. G., Satola, S. W., Crispell, E., Wylie, T., Gao, H., … Fritz, S. A. (2015). Discriminatory Indices of Typing Methods for Epidemiologic Analysis of Contemporary *Staphylococcus aureus* Strains. *Medicine*, *94*(37), e1534.

Romano, R., Lu, D., & Holtom, P. (2006). Outbreak of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Skin Infections Among a Collegiate Football Team 50. *J.Athl.Train.*, *41*(1062–6050 (Print)), 141–145.

Rongpharpi, S. R., Hazarika, N. K., & Kalita, H. (2013). The prevalence of nasal carriage of *Staphylococcus aureus* among healthcare workers at a tertiary care hospital in Assam with special reference to MRSA. *Journal of Clinical and Diagnostic Research*, *7*(2), 257–260.

Rossi, F., Diaz, L., Wollam, A., Panesso, D., Zhou, Y., Rincon, S., … Arias, C. A. (2014). Transferable Vancomycin Resistance in a Community-Associated MRSA Lineage. *New England Journal of Medicine*, *370*(16), 1524–1531.

Ruimy, R., Maiga, A., Armand-Lefevre, L., Maiga, I., Diallo, A., Koumaré, A. K., … Feil, E. J.

(2008). The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. *Journal of Bacteriology*, *190*(11), 3962–3968.

Sakoulas, G., Moise-Broder, P. A., Schentag, J., Forrest, A., Moellering, R. C., Eliopoulos, G. M., & Eliopoulos, G. M. (2004). Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Journal of Clinical Microbiology*, *42*(6), 2398–2402.

Samutela, M. T., Kalonda, A., Mwansa, J., Lukwesa-Musyani, C., Mwaba, J., Mumbula, E. M., … Kwenda, G. (2017). Molecular characterisation of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated at a large referral hospital in Zambia. *The Pan African Medical Journal*, *26*, 108.

Schaumburg, F., Kock, R., Friedrich, A. W., Soulanoudjingar, S., Ngoa, U. A., von Eiff, C., … Becker, K. (2011). Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. *PLoS Neglected Tropical Diseases*, *5*(5), 1–8.

Schwartz, D. C., & Ft Cantor, C. (1964). *Separation of Yeast Chromosome-Sized DNAs by Pulsed Field Gradient Gel Electrophoresis*. *Cell* (Vol. 37).

Selim, H. S., & Abaza, A. F. (2015). Microbial contamination of mobile phones in a health care setting in Alexandria, Egypt. *GMS Hygiene and Infection Control*, *10*, 1–9.

Shallcross, L. J., Fragaszy, E., Johnson, A. M., & Hayward, A. C. (2003). The role of the Panton-Valentine leucocidin toxin in staphylococcal disease : a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *13*(1), 43–54.

Sharma-kuinkel, B. K., Ahn, S. H., Rude, T. H., Zhang, Y., Tong, S. Y. C., Ruffin, F., … Fowler, V. G. (2012). Presence of Genes Encoding Panton-Valentine Leukocidin Is Not the Primary Determinant of Outcome in Patients with Hospital-Acquired Pneumonia Due to *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *50*(3), 848–856.

Sharma, V. K., Hackbarth, C. J., Dickinson, T. M., & Archer, G. L. (1998). Interaction of native and mutant MecI repressors with sequences that regulate mecA, the gene encoding penicillin binding protein 2a in methicillin-resistant staphylococci. *Journal of Bacteriology*, *180*(8), 2160–2166.

Shen, H., Akoda, E., & Zhang, K. (2013). Methicillin-Resistant *Staphylococcus aureus* Carriage among Students at a Historically Black University : A Case Study. *International Journal Of Microbiology*, *2013*, 1–8.

Shore, A. C., Tecklenborg, S. C., Brennan, G. I., Ehricht, R., Monecke, S., & Coleman, D. C. (2014). Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. aureus clones, and increasing multiresistance. *Journal of Clinical Microbiology*, *52*(3), 859–870.

Siegel, J. D., Rhinehart, E., Jackson, M., Chiarello, L., & Healthcare Infection Control Practices Advisory Committee. (2007). Management of multidrug-resistant organisms in health care settings, 2006. *American Journal of Infection Control*, *35*(10 Suppl 2), S165-93.

Siegel, J. D., Rhinehart, E., Jackson, M., & Linda. (2018). *Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings (2007)*. Retrieved from

https://www.cdc.gov/infectioncontrol/pdf/guidelines/isolation-guidelines-H.pdf

Sivaraman, K., Venkataraman, N., & Cole, A. M. (2009). *Staphylococcus aureus* Nasal Carriage and its Contributing Factors. *Future Microbiology*, *4*, 999–1008.

Spiegelman, D., Whissell, G., & Greer, C. W. (2005). A survey of the methods for the characterization of microbial consortia and communities. *Canadian Journal of Microbiology*, *51*(5), 355–386.

Stamper, P. D., Louie, L., Wong, H., Simor, A. E., Farley, J. E., & Carroll, K. C. (2011). Genotypic and phenotypic characterization of methicillin-susceptible *staphylococcus aureus* isolates misidentified as methicillin-resistant *staphylococcus aureus* by the BD geneohm MRSA assay. *Journal of Clinical Microbiology*, *49*(4), 1240–1244.

Strommenger, B., Braulke, C., Heuck, D., Schmidt, C., Pasemann, B., Nübel, U., & Witte, W. (2008). spa typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. *Journal of Clinical Microbiology*, *46*(2), 574–581.

Struelens, M. J., Bauernfeind, A., van Belkum, A., Blanc Cookson L Dijkshoorn, D. B., El Solh, N., Etienne, J., … Witte, W. (1996). *Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems*.

Tenover, F. ., Arbeit, R. ., Goering, R. V, Mickelsen, P. A., Murray, B. E., Persing, D. H., & Swaminathan, B. (1995). *Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing*. *Journal of Clinical Microbiology* (Vol. 33).

Tenover, F. C., Arbeit, R., Archer, G., Biddle, J., Byrne, S., Goering, R., … Pfaller6, M. A. (1994). Comparison of Traditional and Molecular Methods of Typing Isolates of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *32*(2), 407–415.

Tenover, F. C., Arbeit, R. D., & Goering, R. V. (1997). How to Select and Interpret Molecular Strain Typing Methods for Epidemiological Studies of Bacterial Infections: A Review for Healthcare Epidemiologists. *Infection Control and Hospital Epidemiology*, *18*(6), 427–439.

Tenover, F. C., Arbeit, R. D., Goering, R. V, Mickelsen, P. A., Murray, B. E., Persing, D. H., & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology*, *33*(9), 2233–2239.

Tenover, F. C., & Goering, R. V. (2009). Methicillin-resistant *Staphylococcus aureus* strain USA300 : origin and epidemiology. *Journal of Antimicrobial Chemotherapy*, *64*, 441–446.

Tong, A., Tong, S. Y. C., Zhang, Y., Lamlertthon, S., Sharma-kuinkel, B. K., Rude, T., … Jr, V. G. F. (2012). Panton-Valentine Leukocidin Is Not the Primary Determinant of Outcome for *Staphylococcus aureus* Skin Infections : Evaluation from the CANVAS Studies. *PLoS ONE*, *7*(5), 1–7.

Tong, S. Y. ., Chen, L. ., & Fowler Jr, V. . (2012). Colonization, Pathogenicity, Host Susceptibility and Therapeutics for *Staphylococcus aureus*: What is the Clinical Relevance? *Semin Immunopathol*, *34*(2), 185–200. https://doi.org/10.1007/s00281-011-0300-x.Colonization

Trindade, P. A., McCulloch, J. A., Oliveira, G. A., & Mamizuka, E. M. (2003). Molecular

techniques for MRSA typing: current issues and perspectives. *Brazilian Journal of Infectious Diseases*, *7*(1), 32–43.

van Belkum, A., Verkaik, N.-J., de Vogel, C.-P., Boelens, H. ., Verveer, J., Nouwen, J.-L., … Wertheim, H.-F. (2009). Reclassification of *Staphylococcus aureus* Nasal Carriage Types. *The Journal of Infectious Diseases*, *199*(12), 1820–1826.

Van Der Zee, A., Verbakel, H., Van Zon, J. C., Frenay, I., Van Belkum, A., Peeters, M., … Bergmans, A. (1999). Molecular genotyping of *Staphylococcus aureus* strains: Comparison of repetitive element sequence-based PCR with various typing methods and isolation of a novel epidemicity marker. *Journal of Clinical Microbiology*, *37*(2), 342–349.

van Hal, S. J., Lodise, T. P., & Paterson, D. L. (2012). The Clinical Significance of Vancomycin Minimum Inhibitory Concentration in *Staphylococcus aureus* Infections: A Systematic Review and Meta-analysis. *Clinical Infectious Diseases*, *54*(6), 755–771.

Vandendriessche, S., Vanderhaeghen, W., Larsen, J., de Mendonça, R., Hallin, M., Butaye, P., … Denis, O. (2014). High genetic diversity of methicillin-susceptible *Staphylococcus aureus* (MSSA) from humans and animals on livestock farms and presence of SCCmec remnant DNA in MSSA CC398. *Journal of Antimicrobial Chemotherapy*, *69*(2), 355–362.

Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimmo, G. R., Heffernan, H., … Etienne, J. (2003). Community-Acquired Methicillin- Resistant *Staphylococcus aureus* Carrying Panton-Valentine Leukocidin Genes : Worldwide Emergence. *Emerging Infectious Diseases*, *9*(8), 978–984.

Vandenesch F Enright M, Vandenesch F, Naimi T, Enright M, N. T. (2003). Community-

acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worlwwide emergence. *Emerging Infectious Diseases*, *9*(8), 978–984.

Walia, S. S., Manchanda, A., Narang, R. S., Anup, N., Singh, B., & Kahlon, S. S. (2014). Cellular telephone as reservoir of bacterial contamination: Myth or fact. *Journal of Clinical and Diagnostic Research*, *8*(1), 50–53.

Watkins, R. R., Lemonovich, T. L., & File, T. M. (2012). An evidence-based review of linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): Place in therapy. *Core Evidence*, *7*, 131–143.

Welsh, J., & McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, *18*(24), 7213–7218.

Wenzel, R. P. (2004). The Antibiotic Pipeline ? Challenges, Costs, and Values. *New England Journal of Medicine*, *351*(6), 523–526.

Wertheim, H. F. L., Walsh, E., Choudhurry, R., Melles, D. C., Boelens, H. A. M., Miajlovic, H., … Van Belkum, A. (2008). Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. *PLoS Medicine*, *5*(1), 0104–0112.

Wielders, C. L. C., Fluit, A. C., Brisse, S., Verhoef, J., & Schmitz, F. J. (2002). mecA gene is widely disseminated in *Staphylococcus aureus* population. *Journal of Clinical Microbiology*, *40*(11), 3970–3975.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids*

Research, *18*(22), 6531–6535. Retrieved from

Wong, H., Louie, L., Lo, R. Y. C., & Simor, A. E. (2010). Characterization of *Staphylococcus aureus* isolates with a partial or complete absence of staphylococcal cassette chromosome elements. *Journal of Clinical Microbiology*, *48*(10), 3525–3531.

Wongwanich, S., Tishyadhigama, P., Paisomboon, S., & Ohta, T. (2000). Epidemiological analysis of Methicillin-resistant *Staphylococcus aureus* in Thailand. *Southeast Asian J T Rop Med Public Health*, *31*(1), 72–76.

Yildirim, M., Sahin, I., Basak, S., Oksuz, S., Ozaydin, C., Acar, S., … Karabay, O. (2007). The Investigation of Nasal MRSA Carriage and Colonization of Nasopharyngeal Pathogens at a Primary School in Düzce. *Turk J Med Sci*, *37*(6), 359–365.

Yoon, Y. K., Park, D. W., Sohn, J. W., Kim, H. Y., Kim, Y.-S., Lee, C.-S., … Kim, M. J. (2014). Multicenter prospective observational study of the comparative efficacy and safety of vancomycin versus teicoplanin in patients with health care-associated methicillin-resistant *Staphylococcus aureus* bacteremia. *Antimicrobial Agents and Chemotherapy*, *58*(1), 317–324.

Yoong, P., & Torres, V. J. (2013). The effects of *Staphylococcus aureus* leukotoxins on the host: The effects of *Staphylococcus aureus* leukotoxins on the host: cell lysis and beyond. *Curr Opin Microbiol*, *16*(1), 63–69. https://doi.org/10.1016/j.mib.2013.01.012.The

Zakai, S. A. (2015). Prevalence of methicillin-resistant *staphylococcus aureus* nasal colonization among medical students in Jeddah, Saudi Arabia. *Saudi Medical Journal*, *36*(7), 807–812.

Zhang, K., Mcclure, J., Elsayed, S., Louie, T., & Conly, J. M. (2005). Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome. *Microbiology*, *43*(10), 5026–5033.

APPENDICES

Appendix I: Recruitment information sheet

Dear participant,

Thank you for enrolling in this study. This letter is intended to bring to you attention the details of the study as regards your participation.

The title of the study reads as below:

Carriage of methicillin-resistant *staphylococcus aureus* (MRSA) among students of Jomo Kenyatta University of Agriculture and Technology

The objectives of the study include:

1. To isolate and determine antimicrobial resistance patterns associated with the *Staphylococcus aureus* strains isolated from anterior nares, pens, and mobile phones of students residing in hostels at the Jomo Kenyatta University of Agriculture and Technology.
2. To determine the prevalence of MRSA among the *Staphylococcus aureus* isolates obtained from these participants.
3. To determine the presence of *mecA* gene among detected MRSA
4. To identity possible factors that may be associated with carriage of nasal MRSA

The principal investigator is Khasabuli Y. Osborn, an MSC student at the Institute of Tropical Medicine and Infectious Diseases (ITROMID).

The samples for this study will be collected at the Jomo Kenyatta University of Agriculture and Technology and analysis will be conducted at the Kenya Medical Research Institute (KEMRI).

Upon your voluntary decision to participate in this study, you are required to sign the **CONSENT FORM,** which will be provided to you. Prior to signing, please ensure that you read all the information provided.

Thank you.

Appendix II: Consent form

Title of the project: Carriage of methicillin-resistant *staphylococcus aureus* (MRSA) among students of Jomo Kenyatta University of Agriculture and Technology

Investigator: Khasabuli Y. Osborn.

I.D. TM302-2128/2014

Institution affiliation: Master of Science student in Medical microbiology

Institute of Tropical Medicine and Infectious Diseases

(ITROMID)

Sponsor: Kenya Medical Research Institute

What is this study about?

I am a Master's degree student at ITROMID doing a Master of Science in Medical microbiology. I am conducting a research on the carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among students residing in university hostels. Your participation in this research is voluntary.

Why is this study being done?

This research is aimed at detecting circulating MRSA in university facilities. MRSA is associated with causation of skin and soft tissue infections like boils that create a lot of

discomfort especially while concentrating to read. If no treatment is undertaken, more dangerous infections that are life threatening like meningitis may result.

What will happen to me in this study?

Cotton wool swabs will also be collected from your nostrils, pens and mobile phones. You will also fill a questionnaire that will be administered to you by the moderators.

You will not write your name on the questionnaire for the purpose of confidentiality. Your questionnaires will have unique codes, which will also be tagged in all you samples.

What do I gain?

After completion of the study, results and recommendations regarding possible strategies of preventing further spread of MRSA infections will be made available to the university library where you can access them.

Will I be harmed?

There will be no harm whatsoever. However, an unpleasant sensation may develop due to swabbing. When this occurs, you are advised to on rub gently the outside of their nostrils following the completion of the procedure to alleviate the sensation.

Who else will know about my information?

The person who will have the right of access to this information will be any person of your choice and me. The laboratory results will not be revealed alongside your identity.

Who can I contact in case I have questions?

You are allowed to communicate to either your moderator or me at any time of the day during data collection. You are free to share any issue that requires immediate attention at your own convenience.

My contacts are as follows:

Phone number: 0726520491

Email: khasabuli10@gmail.com

Can I contact anyone independent of the study?

You are allowed to seek for any consultation on legal matters regarding your participation in this study.

Do I get paid?

As a participant, you will not be entitled to monetary reimbursement.

Will my samples be stored, exported, or used for future studies?

Your samples will be transported immediately to KEMRI for analyses. Storage of samples after analysis will depend on your choice as indicated below. Please tick appropriately.

Indicate your choice below:

___ I agree to allow my samples to be kept and used for future research on MRSA

___ I do not agree to allow my samples to be kept and used for future research on MRSA

CONSENT AND SIGNATURE OPTIONS:

I have read the consent and understood all that is required of me and therefore I accept to participate in this study fully.

Signature of participant _____

Date

Signature of witness _____

Date

Signature of principal investigator _____

Date

Appendix III: Questionnaire form

Date _____

Study number _____

Sex

a) Male ☐

b) Female ☐

Age

a) 18-30 years ☐

b) 30-40 years ☐

1. How many students are you in your hostel room?

   a) One ☐

   b) Two ☐

   c) Three ☐

   d) Four ☐

2. When was the last time you were hospitalized?

   a). Less than 48 hours ago ☐

   b). More than 48 hours ago ☐

3. Do you participate in any sporting activities?

   a) Yes. ☐

   b) No ☐

4. If YES, Please specify the type of sporting activity.

5. Do you disinfect you pen or mobile phone

   a) Yes ☐

b) No ☐

c) If Yes, how often?

i) Always ☐

ii) Rarely ☐

6. Do you share your pen and/or mobile phone with your classmates?

a) Yes ☐

b) No ☐

7. Do you share other personal effectives like sheets, towels, toothbrushes or clothes?

a) Yes ☐

b) No ☐

8. How do you clean your nostrils?

a) Using a handkerchief ☐

b) Using my fingers ☐

c) By other means ☐

d) Never clean ☐

9. Do you use antibacterial soaps while bathing or washing clothes?

a) Yes ☐

b) No. ☐

Appendix IV: Procedures

Gram staining

1. A heat-fixed smear of bacterial cells was flooded with crystal violet and air dried for one minute.
2. The slide was washed in a gentle and indirect stream of tap water for 2 seconds.
3. The slides was flooded with Gram's iodine and left for 1 minute.
4. The slide was washed in a gentle and indirect stream of tap water for 2 seconds.
5. Acetone alcohol decolorizer was added to the slide drop by drop until the decolorizing agent running from the slide cleared.
6. The slide was flooded with a counterstain, safranin, and left for 1 minute.
7. The slide was washed in a gentile and indirect stream of tap water until no colour was seen in the effluent and then blot dried using an absorbent paper.
8. The results of the staining procedure were observed under oil immersion (×100) using a bright field microscope.

Coagulase test

1. A Staphylococcal colony was emulsified in a drop of water on a clean glass slide with minimal spreading
2. Similar emulsifications were made for positive and negative control strains
3. A sterile straight wire was dipped into undiluted plasma at room temperature, withdrawn, and the adhered plasma stirred into the staphylococcal suspension. The wire was flamed and the same procedure repeated for positive and negative control strains

4. A coarse clumping of cocci visualized with the naked eye within 10 seconds was read as positive. Absence of clumping or any reaction occurring after 10 seconds was read as negative.

Tube coagulase test

1. A 1-in-6 plasma dilution was prepared in saline (0.85% NaCl) and placed in 1 ml volumes of diluted plasma in small tubes.
2. Colonies of isolated *S. aureus* were emulsified in 1 ml of diluted rabbit plasma until a milky suspension was visualized.
3. The tubes were incubated in water bath for 4 hours.
4. Examination of the tubes for clotting by tilting of the tubes through 90° was done at the 1^{st}, 2^{nd} and 4^{th} hour.
5. Negative tubes were left at room temperature over-night for re-examination.

Catalase test

1. A small amount of bacterial colony was transferred to a surface of a clean glass slide using a sterile wooden stick.
2. A drop of 3% H_2O_2 was placed on the slide and mixed.
3. A rapid evolution of oxygen with the first 5-10 seconds was read as a positive result.
4. Absence of bubbles within the first 5-10 seconds was read as a negative result.
5. The glass slide was disposed in a biohazard glass disposal container.

Appendix V: Ethical approval letter from KEMRI

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **September 30, 2015**

TO: **OSBORN Y. KHASABULI,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR,**
NAIROBI

Forwarded 6/10/2015

Dear Sir,

RE: PROTOCOL NO. KEMRI/SERU/CMR/0024/3123 (*RESUBMISSION-INITIAL SUBMISSION*): CARRIAGE OF METHICILLIN-RESISTANT STAPHYLOCCOCCUS AUREAUS (MRSA) AMONG STUDENTS OF JOMO KENYATTA UNVIERSITY OF AGRICULTURE AND TECHNOLOGY.

Reference is made to your letter dated 17th September, 2015. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on 21st September, 2015.

This is to inform you that the Committee notes that the issues raised during the 242nd meeting of the KEMRI/Ethics Review Committee (ERC) held on 18th August, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **30th September, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **September 29, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **August 19, 2016**.

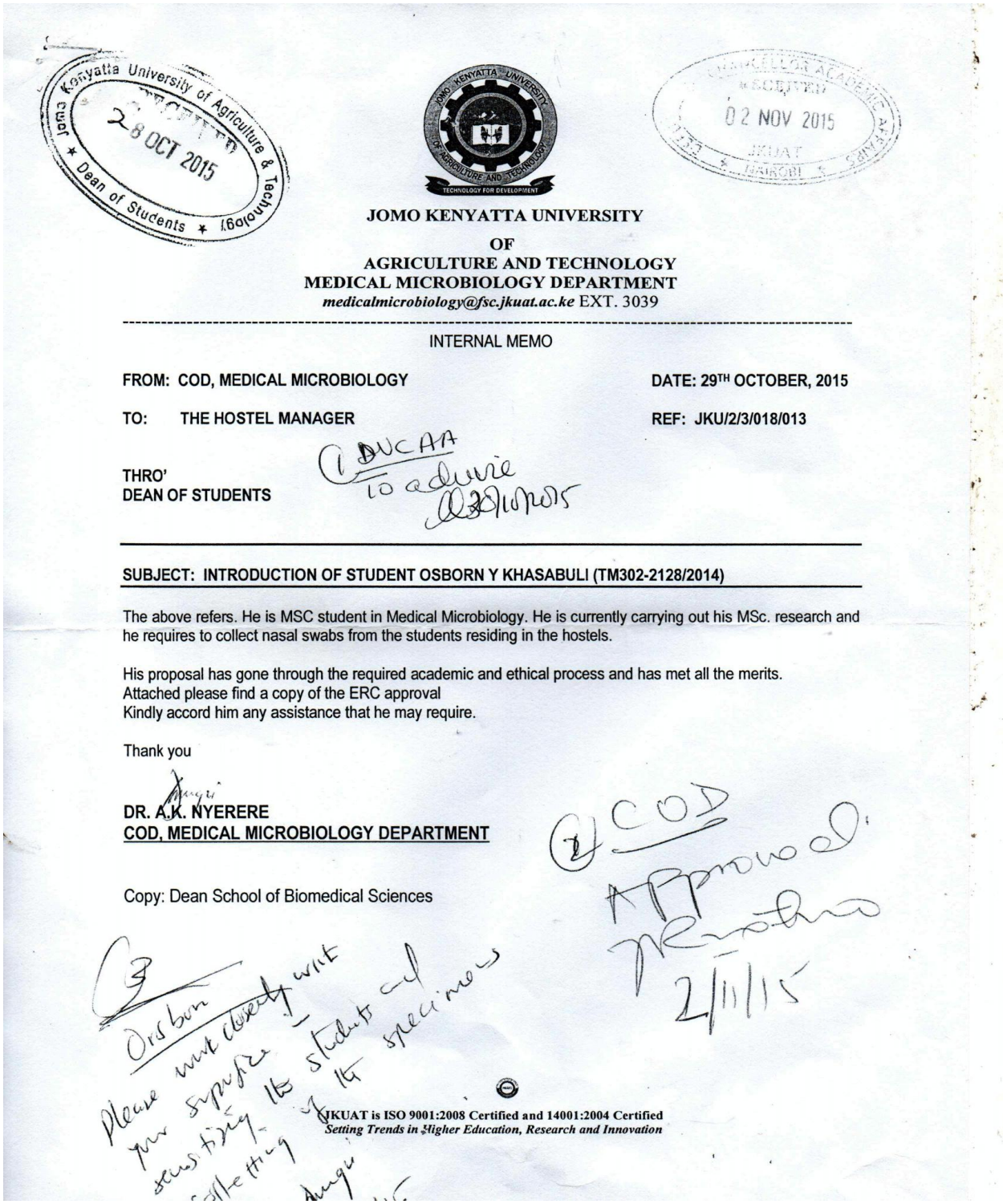
You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

FOR:
PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix VI: Approval letter from the JKUAT administration



Jomo Kenyatta University of Agriculture & Technology
Dean of Students
28 OCT 2015
RECEIVED

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
TECHNOLOGY FOR DEVELOPMENT

VICE CHANCELLOR ACADEMIC AFFAIRS
RECEIVED
02 NOV 2015
JKUAT
NAIROBI

JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
MEDICAL MICROBIOLOGY DEPARTMENT
medicalmicrobiology@fsc.jkuat.ac.ke EXT. 3039

INTERNAL MEMO

FROM: COD, MEDICAL MICROBIOLOGY **DATE: 29TH OCTOBER, 2015**

TO: THE HOSTEL MANAGER **REF: JKU/2/3/018/013**

THRO'
DEAN OF STUDENTS

(1) DVCAA to advise 29/10/2015

SUBJECT: INTRODUCTION OF STUDENT OSBORN Y KHASABULI (TM302-2128/2014)

The above refers. He is MSC student in Medical Microbiology. He is currently carrying out his MSc. research and he requires to collect nasal swabs from the students residing in the hostels.

His proposal has gone through the required academic and ethical process and has met all the merits.
Attached please find a copy of the ERC approval
Kindly accord him any assistance that he may require.

Thank you

DR. A.K. NYERERE
COD, MEDICAL MICROBIOLOGY DEPARTMENT

(2) COD Approved 2/11/15

Copy: Dean School of Biomedical Sciences

Osborn Please visit clearly with your supervisors in sensitizing the students and the specimens collecting

JKUAT is ISO 9001:2008 Certified and 14001:2004 Certified
Setting Trends in Higher Education, Research and Innovation

Appendix VII: Analysis of possible factors for *S. aureus* colonization among surveyed students (part A)

Factors	P value, OR, CL at 95% CI						
	Nostril	**phone**	**Pen**	**Nostril and phone**	**Nostril and pen**	**Phone and pen**	**All**
Being male or female	P= 0.02, OR= 0.5, [0.30-0.97]	P= 0.13, OR= 0.67, [0.38-1.17]	P= 0.32, OR= 0.75, [0.41-1.38]	P= 0.10, OR= 0.57, [0.27 – 1.18]	P= O.79, OR= 0.89, [0.39-2.09]	P= 0.57, OR= 0.78 [0.34-1.76]	P= 0.84, OR= 1.11, [0.37-3.28]
Staying in either hall F, E,D,C,B or A	P= 0.008	P= 0.27	P= 0.79	P= 0.16	P= 0.81	P= 0.83	P= 0.35
Staying in fours or in threes and twos	P= 0.003, OR= 2.36 , CI= 1.28 -4.39	P= 0.21 , OR= 1.41, CI= 0.79-2.52	P= 0.48, OR= 1.23, CI= 0.66-2.29	P= 0.05, OR= 2.05, CI= 0.94-4.54	P= 0.84, OR= 1.08, CI= 0.46-2.55	P= 0.36, OR= 1.44 , CI=	P= 0.50 , OR= 1.42 , CI=
Participating or not participating in sports	P= 0.17, OR= 0.67, CI=	P= 0.86, OR= 1.05 , CI=	P= 0.70, OR= 0.89 , CI=	P= 0.50, OR= 0.78 , CI=	P= 0.61, OR= 1.23, CI=	P= 0.53, OR= 1.28 , CI=	P= 0.23, OR= 1.83 , CI=

Appendix VIII: Association of participation in sports and colonization or contamination by *S. aureus* based on gender

Factor	P value, OR, CL at 95% CI						
	Nostril	**Phone**	**Pens**	**Nostrils and phone**	**Nostril and pen**	**Phone and pen**	**All sites**
Participated in sports	P= 0.03, OR= 3.05 (0.99-9.59)	P= 0.003, OR= 4.33 [1.54-12.48]	P= 0.001, OR= 7.09 [1.91-27.91]	P= 0.29, OR= 2.11 [0.43-10.54]	P= 0.06, OR= 5.33 [0.81-39.86]	P= 0.27, OR= 2.10 [0.50-9.95]	P= 0.64, OR= 2.08 [0.20-23.55]
Did not participate in sports							

Appendix IX: Association of participation in sports and colonization or contamination by *S. aureus* based on type of accommodation (those who stayed in fours and those who stayed in less than four per room)

Factor	P value, OR, CL at 95% CI						
	Nostril	**Phone**	**Pens**	**Nostrils and phone**	**Nostril and pen**	**Phone and pen**	**All sites**
Stayed in fours per room	P= 0.05, OR= 0.37 [0.12-1.14]	P= 0.002, OR= 0.23 [0.08-0.65]	P= 0.01, OR= 0.25 [0.07-0.85]	P= 0.29, OR= 0.47 [0.10-2.31]	P= 0.12, OR= 0.22 [0.03-1.40]		P= 0.34, OR= 0.29 [0.02-3.37]
Stayed in less than four per room							

Appendix X: Analysis of possible factors for *S. aureus* colonization and contamination among surveyed students (part B)

	P value, OR, CL at 95% CI						
	Nostril	**phone**	**Pen**	**Nostril and phone**	**Nostril and pen**	**Phone and pen**	**All**
Disinfecting or not disinfecting mobile phones and pens	P= 1.00, OR= 1.00 (0.24-3.82)	P= 0.80, OR= 0.86 (0.21-3.26)	P= 0.82, OR= 0.86 (0.18-3.61)	P= 0.69, OR= 0.42 (0.20-3.30)	P= 1.00, OR= 0.64 (0.03-5.12)	P= 1.00, OR= 0.59 (0.03-4.71)	P= 1.00, OR= 0 (0.00-5.79)
Sharing on not sharing mobile phones and pens	P= 0.78, OR= 1.27 (0.35-4.98)	P= 0.27, OR= 2.22 (0.55-10.33)	P= 1, OR= 0.96 (0.26-3.80)	P= 0.47, OR= 2.84 (0.37-59.84)	P= 0.39, OR= 0.48 (0.11-2.35)	P= 0.70, OR= 2.02 (0.26-42.86)	P= 1.00, OR= 1.01 (0.12-21.85)
Sharing or not sharing personal effects	P= 0.75, OR= 1.11 (0.57-2.17)	P= 0.51, OR= 1.23 (0.64-2.36)	P= 0.53, OR= 0.80 (0.38-1.67)	P= 0.09, OR= 1.85 (0.84-4.05)	P= 0.36, OR= 0.63 (0.20-1.85)	P= 0.84, OR= 0.91 (0.33-2.39)	P= 1.00, OR= 0.97 (0.25-3.83)
Method of cleaning nostrils	P= 0.83, OR= 0.89 (0.29-2.84)	P= 0.93, OR= 1.05 (0.34-3.33)	P= 0.41, OR= 1.87 (0.48-8.51)	P= 1.00, OR= 0.97 (0.23-4.50)	P= 0.24, OR= (nd)	P= 0.47, OR= 0.68 (0.17-3.19)	P= 0.62, OR= (nd)
Washing hands using medicated or non medicated soap	P= 0.70, OR= 0.90 (0.50-1.63)	P= 0.19, OR= 1.44 (0.80-2.62)	P= 0.59, OR= 1.76 (0.62-2.22)	P= 0.23, OR= 1.56 (0.71-3.47)	P= 0.72, OR= 1.16 (0.48-2.85)	P= 0.29, OR= 1.55 (0.64-3.85)	P= 0.30, OR= 2.05 (0.60-7.71)

*nd- The Odds Ratio could not be determined

Appendix XI: Analysis of possible demographic factors associated with antimicrobial resistance (part A)

Factors	P value, OR, CL at 95% CI										
	AMP	**AMC**	**FOX**	**C**	**E**	**CN**	**CIP**	**F**	**SXT**	**NOR**	**LZD**
Being male or female	P= 0.23, OR= 0.67 (0.31-1.42)	P= 1.00, OR= 0.00 (0..00-23.27)	P= 0.37, OR= 0.67 (0.26-1.70)	P= 1.00, OR= 1.34 (0.13-13.58)	P= 0.03, OR= 0.44 (0.19-1.01)*	P= 0.24, OR= 0.21 (0.01-1.82)	P= 1.00, OR= 0.94 (0.15-3.93)	P= 1.00, OR= (nd)	P= 0.02, OR= 1.62 (0.71-3.65)	P= 0.65, OR= 0.79 (0.24-2.46)	P= 1.00, OR= (nd)
Staying in either hall F, E, D, C, B or A	P= 0.16	P= 0.60	P= 0.38	P= 0.70	P= 0.32	P= 0.12	P= 0.80	P= 1.00	P= 0.02*	P= 0.57	P= 1.00
Staying in either twos, threes or fours per room	P= 0.07	P= 0.60	P= 0.32	P= 0.85	P= 0.03*	P= 0.01*	P= 0.15	P= 1.00	P= 0.37	P= 0.50	P= 1.00
Participating or not participating in sports	P= 0.02, OR= 0.428 (0.20-0.93)*	P= 0.32, OR (nd)	P= 0.25, OR. 1.59 (0.67-3.78)	P= 1.00, OR= 0.70 (0.03-7.76)	P= 0.27, OR= 0.64 (0.26-1.51)	P= 1.00, OR= 0.84 (0.11-5.07)	P= 0.71, OR= 1.29 (0.24-6.40)	Na	P= 0.02, OR= 2.43 (1.07-5.53)*	P= 0.63 OR= 1.30 (0.40-4.06)	Na

Na- statistical association could not be determined, nd- The OR could not be determine, * - significant association

Appendix XII: Analysis of possible demographic factors associated with antimicrobial resistance (part B)

Factors	P value, OR, CL at 95% CI										
	AMP	**AMC**	**FOX**	**C**	**E**	**CN**	**CIP**	**F**	**SXT**	**NOR**	**LZD**
Disinfecting or not disinfecting mobile phones	P= 0.37, OR= nd	P= 1.00, OR= 0.00 (0.00-420.10)	P= 0.01, OR= 6.03 (1.30-26.85)*	P= 0.01, OR= 27.38 (2.35-328.87]*	P= 0.66, OR= 1.33 [0.19-7.20]	P= 0.002, OR= 23.25 [3.31-164.54]*	P= 1.00, OR= 0.00 [0.00-57.38]	0 (0%)	P= 37, OR= 0.00 [0.00-3.29]	P= 1.00, OR= 0.00 [0.00-7.46]	0 (0%)
Sharing or not sharing mobile phones	P: 0.69, OR= 1.18 [0.17-6.22]	P= 1.00, OR= nd	P= 1.00, OR= 1.82 [0.16-27.88]	P= 018, OR= 0.14 [0.01-3.78]	P= 1.00, OR= 1.96 [0.24-42.11]	P= 0.04, OR= 0.11 [0.01-0.91]*	P= 1.00, OR= nd	0 (0%)	P= 0.37, OR= nd	P= 1.00. OR= nd	0 (0%)
Sharing or not sharing personal effects	P= 0.09, OR= 2.34 [0.81-7.26]	P= 0.25, OR= nd	P= 0.78, OR= 1.14 [0.41-3.09]	P= 0.57, OR= 0.00 [0.00-4.73]	P= 0.72, OR= 1.16 [0.48-2.73]	P= 1.00, OR= 0.50 [0.02-4.33]	P= 0.68, OR= 0.43 [0.02-3.57]	0 (0%)	P= 0.01, OR= 3.54 [1.33-9.49]*	P= 1.00, OR= 1.02 [0.26-3.61]	0 (0%)
Using handkerchief or fingers and other means for nostril cleaning	P= 0.02, OR= 3.56 [1.06-11.69]*	P= 1.00, OR= nd	P= 0.70, OR= 0.90 [0.17-5.98]	P= 1.00, OR= nd	P= 0.001, OR= 0.17 [0.05-0.54]*	P= 0.40, OR= 0.43 [0.05-10.11]	P= 0.53, OR= 0.51 [0.06-11.63]	P= 1.00	P= 1.00, OR= 1.14 [0.23-7.63]	P= 1.00, OR= 1.13 [0.14-24.34]	P= 1.00, OR= nd
Using medicated or non-medicated soap for hand washing	P= 0.003, OR= 2.86 [1.32-6.23]*	P= 1.00, OR= nd	P= 0.07, OR= 2.53 [0.85-8.00]	P= 1.00, OR= 1.66 [0.15-42.23]	P= 0.42, OR= 1.36 [0.60-3.14]	P= 0.046, OR= nd *	P= 0.72, OR= 1.68 [2.30-12.33]	0 (0%)	P= 0.59, OR= 1.25 [0.53-3.01]	P= 0.18, OR= 2.52 [0.64-11.50]	0 (0%)

*- Significant association, nd- The Odds Ration could not be determined

Appendix XIII: Comparison of MRSA and MSSA strains in regards to the carriage of *lukFS-PV* gene

Strain type/gene	*lukFS-PV* positive	*lukFS-PV* gene negative	Chi Square	d.f.	P value
MRSA	7	19	0.483	1	0.487
MSSA	10	18			

Appendix XIV: Analysis of possible factors associated with the colonization and contamination by MDR and MRSA strains (part A)

Factors	Total number of *S. aureus* isolates (N)	P value, odds ratio and CL at 95% CI	
		MDR	MRSA
Male gender	99	p= 0.19, OR= 0.42 [0.11-1.47]	p= 0.37, OR= 0.68 [0.26-1.70]
Female gender	132		
Residing in halls E, C and B	93	p= 0.06, OR= 2.65 [0.85-8.57]	p= 0.03, OR= 2.46 [0.98-6.25]*
Residing in halls F, D and A	138		
Staying in twos per room	80	P= 0.28, OR= 0.41 [0.09-1.62]	P= 0.38, OR= 0.67 [0.24-1.78]
Staying in threes and fours	151		
Participating in sports	74	P= 0.63= OR= 1.30 [0.40-4.06]	P= 0.77, OR= 1.14 [0.442-2.89]
Not participating in sports			

*- Significant association

Appendix XV: Analysis of possible factors associated with the colonization and contamination by MDR and MRSA strains (part b)

Factors	Total number of *S. aureus* isolates (N)	P value, odds ratio and CI at 95% CL	
		MDR	**MRSA**
Disinfecting mobile phone and pen	10	P= 1.00, OR= 0.79 [0.04-6.92]	P= 0.02, OR= 6.03 [1.30-26.86]
Not disinfecting mobile phone and pen	221		
Sharing mobile phones and pens	220	P= 0.55, OR= 0.73 [0.09-16.28]	P= 1.00, OR= 1.28 [0.16-27.88]
Not sharing mobile phones and pens	11		
Sharing personal effects	57	P= 0.001, OR= 0.02 [0.003-0.084]*	P= 0.78, OR= 1.14 [0.41-3.09]
Not sharing personal effects	174		
Using a handkerchief to clean nostrils	215	P= 1.00, OR= 1.125 [0.138-24.342]	P= 0.70= OR= 0.88 [0.17-5.98]
Using fingers and other means to clean nostrils	16		
Using medicated soaps for washing hands	149	p= 0.18, OR= 2.52 [0.64-11.50	p= 0.08, OR= 2.53 [0.85-8.00]
Using non-medicated soap for washing hands	82		

*- Significant association

Appendix XVI: Manuscript

East African Medical Journal Vol. 94 No. 7 July 2017
CARRIAGE, ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENETIC DIVERSITY OF *STAPHYLOCOCCUS AUREUS* AND MRSA ISOLATES RECOVERED FROM STUDENTS IN A KENYAN UNIVERSITY
Osborn Y. Khasabuli, Caroline Ngugi, Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000-00200, Nairobi, John Kiiru, Kenya Medical Research Institute, P.O Box 19464-00202, Nairobi

CARRIAGE, ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENETIC DIVERSITY OF *STAPHYLOCOCCUS AUREUS* AND MRSA ISOLATES RECOVERED FROM STUDENTS IN A KENYAN UNIVERSITY

O. Y. Khasabuli, C. Ngugi and J. Kiiru

ABSTRACT

Objective: To determine the carriage, antimicrobial susceptibility profiles and genetic diversity of *Staphylococcus aureus* and MRSA isolates recovered from students in a Kenyan University.
Study design: A cross sectional descriptive study.
Setting: Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya.
Subjects: A total of 237 healthy students residing within the University residence halls were recruited.
Results: A total of 231 *S. aureus* isolates were recovered. All isolates were susceptible to nitrofurantoin and linezolid and resistant in high numbers (194, 81.9%) to ampicillin. Resistances to amoxicillin-clavulanic acid, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacin and trimethoprim-sulfamethoxazole were below 20%. The overall prevalence of MRSA among the study population was 11.3% (26/231). Based on carriage of the *mec* cassettes, the SCC*mec*V (61.5%) was more prevalent among the MRSA followed by SCC*mecII* (53.9%). Carriage of the *lukFS-PV* gene was 26.9% and 35.7% among the MRSA and MSSA strains respectively, and there was no statistical association between the two strains with regard to carriage of the gene (p=0.487). Analysis of genetic relations showed evidence of strain sharing among students.
Conclusion: The study revealed the presence of MRSA strains, which are also multi-drug resistant, circulating among a healthy student population in a university setting within Central Kenya. Therefore, these results indicate the existence of potential risk factors, thus necessitating a comprehensive surveillance on MRSA and studies on control measures to help in curbing the spread of MRSA strains.

INTRODUCTION

Staphylococcus aureus is found on the skin surface and the nostrils of approximately 30% of healthy people (1). Although recognised as a commensal, this bacterium can also cause human infections such as boils and pimples, septicemia and pneumonia. Of all colonisable surfaces, the nasal cavity is the primary "reservoir" although significant colonization has been reported in extra-nasal sites such as the vagina, skin, and the gastrointestinal tract
(2). The usual transmission among humans is person-to-person transmission (3). Fomites such as phones have also been implicated in the spread of *S. aureus* (4,5).

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections occur in any setting, but several factors may facilitate the spread of MRSA. These include crowded living conditions, skin cuts, close skin-to-skin contact, contaminated inanimate surfaces, and lack of proper hygiene (6,7). MRSA continue to develop resistance towards available antibiotics, thus rendering management of infections caused by MRSA problematic (8).
Schoolmates are now considered a risky group for MRSA infections and Universities are ideal settings where spread of MRSA is facilitated by close contact among susceptible individuals (3). Naturally, students tend to live in close proximity and share a lot of material such as pens and phones. Such items could act as potential fomites for transmission. Students suffering from skin and other soft tissue infections may experience a lot of discomfort, stigmatization and often miss class days (9). Although a few studies have focused on college students or students participating in particular sporting activities such as football and athletics, no study has been done to determine the extent of colonization of MRSA among students in any of the Kenyan Universities. As such, the prevalence of MRSA and the occurrence of multi-drug resistant strains in this population remain unknown.

Presently, the frequency of incidence of infections caused by MRSA strains continues to grow in school settings worldwide (3,10). Thus, it is likely that Kenyan University students are also at a risk of MRSA infections. Due to unavailability of information on the prevalence of MRSA colonization among university students, management of MRSA infections among students may be ineffective. Prevalence reports are crucial towards implementation of strategies that can help in preventing the spread of MRSA among high risk groups, and perhaps prevent outbreaks of MRSA infections (11). In order to investigate the colonization of *Staphylococcus aureus*, we collected nasal swabs and swabs from phones and pens of students from a local university. The isolates were analysed for resistance to various antimicrobials, prevalence and genetic basis for the MRSA phenotype, carriage of PVL genes and genetic relatedness.

MATERIALS AND METHODS

Study Area and Study Population

The study was conducted at the main campus of Jomo Kenyatta University of Agriculture and Technology in Kenya, from October 2015 to July 2016. Students who resided within the university hostels and those who agreed to give a written consent for participation were recruited. Non-resident students, those on antibiotics and those who refused to give their written consent were not recruited. Sampling size and sampling technique

A total of 237 participants were recruited. A systematic random sampling procedure was used to randomly select students from six residence halls. The number

of participants selected in each hall was based on a ratio of the total number of rooms in each hall.

Collection of samples

A sterile nasal swab softened using a drop of sterile normal saline was inserted into each nostril and gently rotated to scoop a specimen from nasal secretions. The swabs were labelled using codes unique to each participant. The swabs were also collected from phones and pens (fomites) from each of the recruited students. This was done by swabbing over surface of the fomite using a wet sterile swab. All swabs were transferred to Amies transport media before transportation to the laboratory for analysis within 4 hrs.

Bacterial isolation and identification

Upon arrival in the laboratory, the swabs were enriched into trypticase soy broth (TSB) and incubated for 18 to 24 hours. A 10µl aliquot from the enrichment was inoculated on Mannitol Salt Agar (Oxoid) plates and incubated aerobically at 35°C for 24 hours.

Standard methods of identifying *Staphylococcus aureus* were based on colony morphology, Gram staining, coagulase and catalase tests. In order to confirm the isolates as *S. aureus*, *spa*-PCR typing was done. Pure cultures were prepared and preserved in trypticase soy broth supplemented with 15% glycerol and frozen at -20°C until further analysis.

Determination of antibiotic resistance patterns

The modified Kirby-Bauer disk diffusion method for antibiotic susceptibility testing
was employed using commercial antimicrobial discs and results interpreted based on the CLSI guidelines. The discs included ampicillin (AMP, 10µg), amoxicillin-clavulanic acid (AMC, 30), ciprofloxacin (CIP, 10µg), erythromycin (E, 15µg), gentamicin (CN, 10µg), cefoxitin (FOX, 30µg), linezolid (LZD, 30µg), norfloxacin (NOR, 10µg), nitrofurantoin (F, 300µg), chloramphenicol (C, 30µg) and trimethoprim-sulfamethoxazole (SXT, 25µg). *Staphylococcus aureus* (MRSA) ATCC® 33591 was used as control for disc potency and to check the quality of the media. Identification of methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus*

The MRSA strains were detected based on their susceptibility to cefoxitin. Strains with inhibition zone diameters of ≤21mm were considered as methicillin-resistant while those with inhibition zone diameters of ≥21
mm were considered as methicillin-susceptible. Multi-drug resistance was taken as resistance to any β-lactam and at least three antibiotics from the other classes.

Isolation and storage of DNA

DNA from the *S. aureus* strains was recovered using 10% Chelex solution prepared using 1X TE buffer. Isolated DNA was stored at -20°C for future use.

Screening for *MecA* gene and SCC*mec* typing

Amplification and detection of *mecA* gene and SCC*mec* elements was done on all the *S. aureus* isolates using the polymerase chain reaction (PCR) method. For the *mecA* gene, 4µl of the ready to mix 5x FIREPol®Master Mix (Solis Biodyne), was added to 0.4µl of each primer, 1µl of BSA, 12.2 µl of RNase/DNase free PCR water and 2 µl of sample DNA. The forward and reverse primers sequences were 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and 5'-AGT TCT GCA GTA CCG GAT TTG C-3' respectively. Amplification reactions were set at an initial

denaturation temperature of 95°C for 5 minutes. Thereafter, the preparation was subjected to 30 cycles of denaturation at 94°C for 60 seconds, annealing at 56°C for 60 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 7 minutes. The expected Amplicon size was 533bp.

For SCC*mec*-typing, amplification of SCC*mec* elements was done using primers specific for SCC*mec*I, II, III, IV and V. In every PCR tube, 4µl of the ready to mix 5x FIREPol® Master Mix (Solis Biodyne), 0.4µl of both forward and reverse primers, 1µl of BSA, 12.2 µl of RNase/DNase free PCR water and 2 µl of sample DNA. Amplification reactions were initiated with a denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 min and another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min, ending with a final extension step at 72°C for 10 min. A known control strain was included as a positive control for each PCR set. The primer sequences for the SCC*mec* types were as follows: SCC*mec*I forward primer 5'- GCT TTA AAG AGT GTC GTT ACA GG-3' and reverse primer 5'-GTT CTC TCA TAG TAT GAC GTC C-3'= SCC*mec*II forward primer 5'- CGT TGA AGA TGA TGA AGC G-3' and reverse primer 5'-CGA AAT TGG TTA ATG GAC C-3'= SCC*mec*III forward primer 5'-CCA TAT TGT GTA CGA TGC G-3' and reverse primer 5'-CCT TAG TTG TCG TAA CAG ATC G-3'= SCC*mec*IVa forward primer 5'-GCC TTA TTC GAA GAA ACC G-3' and reverse primer 5'-CTA CTC TTC TGA
AAA GCG TCG-3'= SCC*mec*IVb forward primer 5'-TCT GGA ATT ACT TCA GCT GC-3' and reverse primer 5'-AAA CAA TAT TGC TCT CCC TC-3'= SCC*mec*IVc forward primer
5'- ACA ATA TTT GTA TTA TCG GAG AGC-3' and reverse primer 5'-TTG GTA TGA GGT ATT GCT GG-3'= SCC*mec*IVd forward primer
5'-CTC AAA ATA CGG ACC CCA ATA CA-3' and reverse primer 5'-TGC TCC AGT AAT TGC TAA AG-3'= SCC*mec*V forward primer 5'-GAA CAT TGT TAC TTA AAT GAG GG-3' and reverse primer 5'-TGA AAG TTG TAC CCT TGA CAC C-3'. The expected amplicon sizes were as follows: SCC*mec*I, 613bp= SCC*mec*II, 398bp= SCC*mec*III, 280bp= SCC*mec*IVa, 776bp= SCC*mec*IVb, 493bp= SCC*mec*IVc, 200bp= SCC*mec*IVd, 881bp= SCC*mec*V, 325bp.

Detection of *lukFS-PV* gene

PCR for detection of *lukFS-PV* gene was done using the *lukFS-PV* primer sequences of 5'-ATCATTAGGTAAAATGTACTGGACATGA TCA-3' and 5'-CATCAATGTATTGGATAGCAAAAGC3' respectively. The strains analysed were selected randomly to represent those obtained from all halls of residence and from students of different socio-demographics. In every PCR tube, 4µl of the ready to mix 5x FIREPol® Master Mix (Solis Biodyne), 0.4µl of both forward and reverse primers at a concentration of 10pmol, 1 µl of BSA, 12.2 µl of RNase/DNase free PCR water and 2 µl of sample DNA were added. Amplification reactions were set at an initial denaturation temperature of 94°C for 5 minutes. Thereafter, the preparation was subjected to 30 cycles at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, final extension at 72°C for 7 minutes and a holding temperature set at 4°C. The expected product size was 433 bp. A control strain was included as positive control in all reactions.

Repetitive sequence-based (Rep)-PCR genotyping

Selection of isolates for Rep-typing was made with a view to represent strains from

participants of all socio-demographic characteristics and to represent strains of different antimicrobial resistance patterns. The Staphrep primer with the following base
sequence= 5'-TCGCTCAAAACAACGACACC-3' was used at a concentration of 75pmol per 31.5-µl assay. Amplification reactions were done using the Pure-Taq (Ready-to-go) PCR beads (GE Healthcare), 1.5µl of the Staphrep primer listed in table 1, 25µl of RNase/DNase free PCR water and 5µl of sample DNA. Thermal cycling parameters were set follows: initial denaturation at 95°C for 7 minutes followed by 31 cycles of 94°C for 1 minute, annealing at 40°C for 1 minute, extension at 65°C for 8 minutes and a final extension at 65°C for 15 minutes. A holding temperature was set at 10°C. A control strain was included as a marker. The rep-PCR banding patterns were
analysed using GelCompar II (Biomerieux Company).

Visualization of PCR products
PCR products were visualized under a UV trans illuminator on 1.2% agarose gel loaded with ethidium bromide.

Statistical analysis
Statistical analysis was performed using the SPSS version 21 (IBM Corporation). Chi-square and Fisher exact tests were used for analyses of bivariate variables while odds ratios were used for analyses of the association between nasal carriage and demographic factors. The level of significance was set at 95% CI.

Ethical considerations
An approval to conduct this study was sought from the Kenya Medical Research Institute`s Scientific and Ethics Review Unit. Permission to visit, recruit, interview and sample the students was sought from the University's administration. Also, prior to recruitment, a written consent was obtained from students.

RESULTS

General characteristics of study participants
All participants in this study were aged between 18 and 30 years. Characteristics of these participants are captured in table 1 below. Carriage of S. aureus and possible risk factors for colonization among study participants

A total of 231 isolates were recovered. Of these, 78(33.8%) were obtained from the participants noses while 87(37.7%) and 66(28.6%) were obtained from phones and pens respectively. Carriage of S. aureus with respect to the different variables and their specific categories analyzed in this study is provided in table 2 below.

Nasal carriage of S. aureus was significantly higher among females students 47(40.2%) compared to males 31(26.7%) (p=0.02, OR=0.52, CI=0.29-0.93). However, we also found out that colonization of nostrils was more common among male students who participated in sports than females (p=0.03, OR= 3.05, CI= 0.99-9.59). Analysis also revealed that nasal carriage was significantly higher among participants occupying halls 4 (a female`s hostel) and 3 (a male`s hostel) in comparison to other halls (p=0.001, OR= 3.28 CI= 1.58-6.83). We also established that nasal colonization was significantly higher among those who stayed in groups of four (congested conditions) than in those who stated in lesser numbers (p=0.003, OR= 2.36, CI= 1.28-4.39).

Table 1
Descriptive statistics of the sampled university students, 2016

Variable	Categories	Frequency	Percentage
Gender	Males	120	50.6%
	Females	117	49.4%
Age	18-30 years	237	100%
Resident halls	Hall 1	48	20.3%
	Hall 2	39	16.5%
	Hall 3	13	5.5%
	Hall 4	30	12.7%
	Hall 5	9	3.8%
	Hall 6	98	41.4%
Students per room	In Fours	124	52.3%
	In Threes	11	4.6%
	In Twos	102	43%
Last time of hospitalization	Less than 48 hours ago	0	0%
	More than 48 hours ago	237	100%
Sporting activities	Participated	80	33.8%
	Did not participate	157	66.2%
Disinfection of pens and phones	Disinfected	12	5.1%
	Did not disinfect	225	94.9%
Sharing of phones pens	Shared	223	94.1%
	Did not share	14	5.9%
Sharing of personal effects	Shared	57	24.1%
	Did not share	180	75.9%
Cleaning of nostrils	Used handkerchief	214	90.3%
	Used fingers	9	3.8%
	Used other means	8	3.4%
	Never cleaned	6	2.5%
Washing hands	Used medicated soaps	148	62.4%
	Used non-medicated soaps	89	37.6%

*Type of accommodation is the number of students in a hostel room= personal effects represented items like clothes, bed sheets, towels and tooth brush

Table 2
Carriage of *S. aureus* in the sampled student population

Variable	Categories	Number of students tested	Colonization on a single site			Colonization on multiple sites			
			Nostril n (%)	Phone n (%)	Pens n (%)	Nostrils and phone	Nostril and pen	Phone and pen	All sites
Gender	Males	120	31 (26.7%)	38 (31.7%)	30 (25%)	15 (12.5%)	14 (11.7%)	14 (11.7%)	9 (7.5%)
	Females	117	47 (40.2%)	49 (41.8%)	36 (30.8%)	25 (21.4%)	15 (12.8%)	17 (14.5%)	8 (3.4%)
Resident halls	Hall 1	48	15 (31.1%)	16 (33.3%)	17 (35.4%)	6 (12.5%)	4 (8.3%)	6 (12.5%)	1 (2.1%)
	Hall 2	39	15 (38.5%)	20 (51.3%)	10 (25.6%)	10 (25.6%)	6 (15.4%)	7 (17.9%)	4 (10.3%)
	Hall 3	13	7 (53.8%)	5 (38.5%)	3 (23.1%)	2 (15.4%)	1 (7.7%)	2 (15.4%)	1 (7.7%)
	Hall 4	30	17 (56.7%)	12 (40%)	9 (30%)	9 (30%)	4 (13.3%)	4 (13.3%)	3 (10%)
	Hall 5	9	3 (33.3%)	4 (44.4%)	3 (33.3%)	2 (22.2%)	2 (22.2%)	2 (22.2%)	2 (22.2%)
	Hall 6	98	21 (21.4%)	29 (29.6%)	24 (24.5%)	12 (12.2%)	11 (11.2%)	10 (10.2%)	6 (6.1%)
Occupancy	In Fours	124	52 (41.9%)	49 (39.5%)	37 (29.8%)	25 (20.2%)	17 (13.7%)	20 (16.1%)	11 (8.9%)
	In Threes	11	3 (27.3%)	4 (36.4%)	3 (27.3%)	4 (36.4%)	0 (0%)	0 (0%)	0 (0%)
	In Twos	102	23 (22.5%)	32 (31.4%)	26 (25.5%)	12 (11.8%)	12 (11.8%)	11 (10.8%)	6 (5.9%)
Sporting activities	Participated	80	22 (27.5%)	30 (37.5%)	21 (26.3%)	12 (15%)	11 (13.8%)	12 (15%)	8 (10%)
	Did not participate	157	57 (36.3%)	57 (36.3%)	45 (28.7%)	29 (18.5%)	18 (11.5%)	19 (12.1%)	9 (5.7%)
Disinfection of pens and phones	Disinfected	12	4 (33.3%)	4 (33.3%)	3 (25%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	0 (0%)
	Did not disinfect	225	75 (33.3%)	83 (36.9%)	63 (28%)	40 (17.8%)	28 (12.4%)	30 (13.3%)	17 (7.6%)
Sharing of phones/pens	Shared	223	75 (33.6%)	84 (37.7%)	62 (27.8%)	40 (17.9%)	26 (11.7%)	30 (13.5%)	16 (7.2%)
	Did not share	14	4 (28.6%)	3 (21.4%)	4 (28.6%)	1 (7.1%)	3 (21.4%)	1 (7.1%)	1 (7.1%)

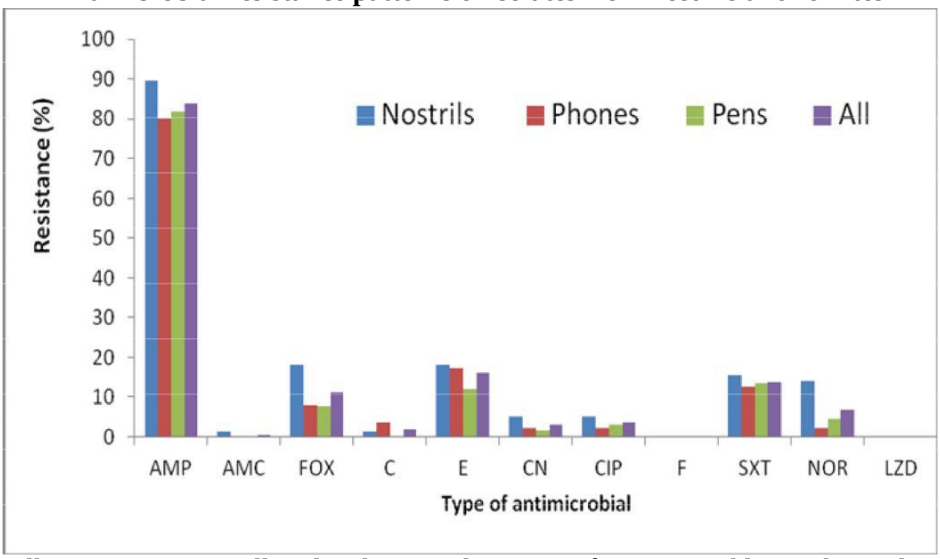
Sharing of personal effects	Shared	57	20 (35.1%)	23 (40.4%)	14 (24.6%)	14 (24.6%)	5 (8.8%)	7 (12.3%)	4 (7%)
	Did not share	180	59 (32.8%)	64 (35.6%)	52 (31.7%)	27 (15%)	24 (13.3%)	24 (13.3%)	13 (7.2%)
Cleaning of nostrils	Used handkerchief	220	72 (32.7%)	80 (36.4%)	63 (28.6%)	38 (17.3%)	29 (13.2%)	28 (12.7%)	17 (7.7%)
	Used fingers	9	3 (33.3%)	3 (33.3%)	1 (11.1%)	2 (22.2%)	0 (0%)	1 (11.1%)	0 (0%)
	Used other means	8	3 (37.5%)	3 (37.5%)	2 (25%)	1 (12.5%)	0 (0%)	2 (25%)	0 (0%)
Washing hands	Used medicated soaps	148	48 (32.4%)	59 (39.9%)	43 (29.1%)	29 (19.6%)	19 (12.8%)	22 (14.9%)	13 (8.8%)
	Used non-medicated soaps	89	31 (34.8%)	28 (31.5%)	23 (25.8%)	12 (13.5%)	10 (11.2%)	9 (10.1%)	4 (4.5%)

*Type of accommodation is the number of students in a hostel room= personal effects represent items like clothes, bed sheets, towels and tooth brush

Antimicrobial resistance profiles of isolates recovered from the student population

The 231 isolates were tested against 11 antimicrobials. In general, we found low resistances to erythromycin (16%), trimethoprim-sulfamethoxazole (13.9%), cefoxitin, (11.3%), norfloxacin (6.9%), gentamicin (3%), ciprofloxacin (3.5%), chloramphenicol (1.8%) and amoxicillin-clavulanic acid (0.4%). However, higher resistances of above 80% were recorded for ampicillin (84%). Most isolates showed high resistance to ampicillin regardless of the source. Amoxicillin-clavulanic acid-resistance was recorded in a single isolate recovered from the nostrils. With regard to the sites of isolation, the prevalence of cefoxitin-resistant isolates also refereed henceforth as MRSA strains was 18% for isolates recovered from nostrils compared to phones (8.1%) and pens (7.6%). In addition, the proportion of isolates from nose that were resistant to gentamicin and ciprofloxacin (both 5.1%), trimethoprim-sulfamethoxazole (15.4%) and norfloxacin (14.1%) was slightly higher than the proportion of isolates recovered from fomites. A high proportion of isolates from nostrils were also resistant to erythromycin (17.2%) compared to from phones (17.2%) and pens (12.1%). Chloramphenicol resistance was high among isolates recovered from phones (3.5%) compared to nostrils (1.3%) while no resistance was recorded for isolates recovered from the pens (Figure 1).

Figure 1
Antimicrobial resistance patterns of isolates from nostrils and fomites

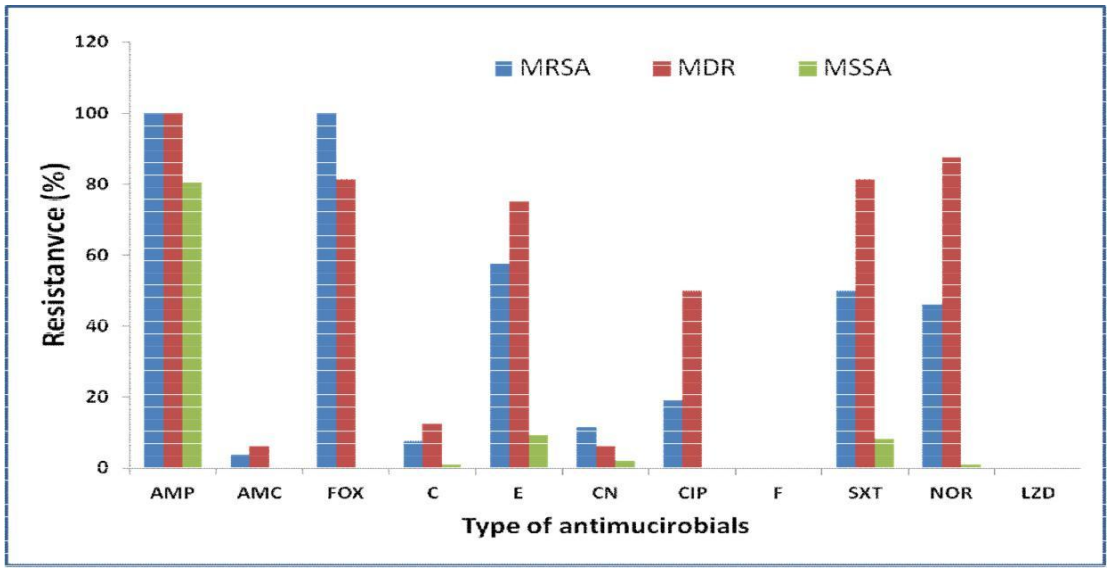


*AMP, ampicillin= AMC, amoxicillin-clavulanic acid= FOX, cefoxitin= C, chloramphenicol= E, erythromycin= CN, gentamicin= CIP, ciprofloxacin= F, nitrofurantoin= SXT, trimethoprim-sulfamethoxazole= NOR, norfloxacin= LZD, linezolid= All, all isolates from nostrils, phones and pens combined.

Resistance phenotypes associated with MRSA, MDR and MSSA strains

Our study revealed that all the MRSA and MDR strains were resistant to ampicillin while 80.5% of the MSSA were susceptible to this antimicrobial. In addition, high resistances (above 40%) were observed for erythromycin, norfloxacin and trimethoprim-sulfamethoxazole among the MRSAs compared to lower resistances of below (10%) for the same antimicrobials among the MSSA. Low resistance levels of less than 20% were recorded for amoxicillin-clavulanic acid, chloramphenicol and gentamicin among the MDRA and MDRS strains. Also, a majority of the MRSAs (57.7%) and MDR strains (75%) showed resistance to erythromycin (Figure 2). We also established that MRSA strains isolates were more likely to display multi-drug resistance compared MSSA strains (P=0.001).

Figure 2
Antimicrobial resistance patters of *S. aureus* isolates obtained from the study population



*MRSA, methicillin-resistant *S. aureus*, MDR, multi-drug resistant, MSSA, methicillin-susceptible *S. aureus*= AMP, ampicillin= AMC, amoxicillin-clavulanic acid= FOX, cefoxitin= C, chloramphenicol= E, erythromycin= CN, gentamicin= CIP, ciprofloxacin= F, nitrofurantoin= SXT, trimethoprim-sulfamethoxazole= NOR, norfloxacin= LZD, linezolid

Prevalence and possible epidemiologic factors associated with carriage of MRSA and MDR strains among study participants

Out of the 231 isolates, 26 (11.3%) were MRSA while 16 (6.93%) were MDRs. We also found that 50% of the MRSAs were also MDRs. The carriage of MRSA and MDRs among the study population is provided in table 3 below.

Our study showed that the gender of participants was not significantly associated with the risk of colonization by MRSAs or MDRs on nostrils or fomites (p>0.05). Isolates recovered from halls two, four and five were more likely to be MRSAs as opposed to those recovered from halls one, three and six (P= 0.03, OR= 2.46, CI= 0.98-6.25). We also

established that those who did not disinfect their phones were more likely to carry an MRSA strain in the nose or on their fomites (P=0.02, OR=6.03, CI= 1.30-26.86).

Table 3
Proportions of MDRs and MRSA recovered from study participants

Variables	Categories	Total number of *S. aureus* isolates (n)	Total number of MDRs, n (%)	Number of MRSA strains, n (%)
Gender	Males	99	4 (4%)	9 (9%)
	Females	132	12 (9.1%)	17 (12.9%)
Hall of residence	Hall one	49	3 (6.1%)	4 (8.2%)
	Hall two	45	4 (8.9%)	5 (11.1%)
	Hall three	15	0 (0%)	1 (6.7%)
	Hall four	38	5 (13.2%)	8 (21.1%)
	Hal five	10	1 (10%)	2 (20%)
	Hall six	74	3 (4.1%)	6 (8.2%)
Type of accommodation	Twos	80	3 (3.8%)	7 (8.8%)
	Threes	12	2 (16.7%)	3 (25%)
	Fours	139	11 (7.9%)	16 (11.5%)
Sporting activities	Participated	74	6 (8.1%)	9 (12.2%)
	Did not participate	157	10 (6.4%)	17 (10.8%)
Disinfection of pens and phones	Disinfected	10	1 (10%)	4 (40%)
	Did not disinfect	221	15 (6.8%)	22 (10%)
Sharing phones and pens	Shared	220	15 (6.8%)	25 (11.4%)
	Did not share	11	1 (9.1%)	1 (9.1%)
Sharing personal effects	Shared	57	2 (3.5%)	7 (12.3%)
	Did not share	174	14 (8.0%)	19 (10.9%)
Cleaning of nostrils	Used a handkerchief	215	14 (6.5%)	24 (11.2%)
	Used fingers	6	0 (0%)	0 (0%)
	Cleaned by other means	10	1 (10%)	2 (20%)
Bathing or washing clothes	Used medicated soaps	149	13 (8.7%)	21 (14.1%)
	Used non-medicated soaps	82	3 (3.7%)	5(6.1%)

**Type of accommodation is the number of students in a hostel room= Personal effects represent items like clothes, bed sheets, towels and tooth brush, MRSA, methicillin-resistant S. aureus, MDR, multi-drug resistant, MSSA, methicillin-susceptible S. aureus*

Prevalence of *spa, mecA,* SCC*mec* elements and *lukFS-PV* genes among the *S. aureus* isolates

All the isolates carried the Spa genes indicating they were *Staphylococcus* strains. As expected, majority (65.4%) of the MRSA harboured the *mecA* gene and 52.9% of these were also MDRs. The most common *mec*-cassette was SCC*mec*V (6.9%) followed by SCC*mec*II (6.5%). SCC*mec*III was present in only 1.3% of the isolates. None of the SCC*mec*IV types was found among the isolates. SCCmecII was highly prevalent among the MDRs (68.8%) than among MRSA strains (53.9%). Similarly, SCC*mec*V was highly prevalent among the MDRs (62.5%) and MRSA (61.5%) compared to MSSA strains, which did not harbour this element. SCC*mec*III was present in a small proportion (11%) of the MRSA strains (Table 4). Carriage of the *lukFS-PV* gene within our isolates was only at 31.5% and only 26.9% of the MRSA and MSSA strains (table 5), and there was no statistical association between the two strains with regard to carriage of the gene (p=0.487).

Table 4
Proportion of *S. aureus* isolates harbouring *spa, mecA*, SCC*mec* elements and *lukFS-PV* genes among the *S. aureus* isolates

Type/source of isolate	Type of gene (N=231)				
	MecA	SCC*mec*I	SCC*mec*II	SCC*mec*III	SCC*mec*V
Overall	17 (7.4%)	7 (3.0%)	15 (6.5%)	3 (1.3%)	16 (6.9%)
MRSA	17 (65.4%)	4(15.4%)	14 (53.9%)	3 (11.5%)	16 (61.5%)
MSSA	0 (0%)	2 (1.0%)	0	0	0
MDR	9 (56.3%)	1 (6.3%)	11 (68.8%)	0 (0%)	10 (62.5%)
Nostril	8 (10.3%)	3 (3.9%)	10 (12.8%)	2 (2.6%)	10 (12.8%)
Fomites	9 (5.9%)	4 (2.6%)	5 (3.3%)	1 (0.7%)	6 (3.9%)

**The prevalences for SCCmec Iva, SCC mec IVb, SCC mec IVc and SCC mec IVd were 0%, and therefore, were not included in the table= MRSA, methicillin-resistant S. aureus= MDR, multi-drug resistant= MSSA, methicillin-susceptible S. aureus= Overall, all the isolates*

Table 5
Proportion of *S. aureus* isolates harbouring *lukFS-PV* genes *S. aureus* isolates

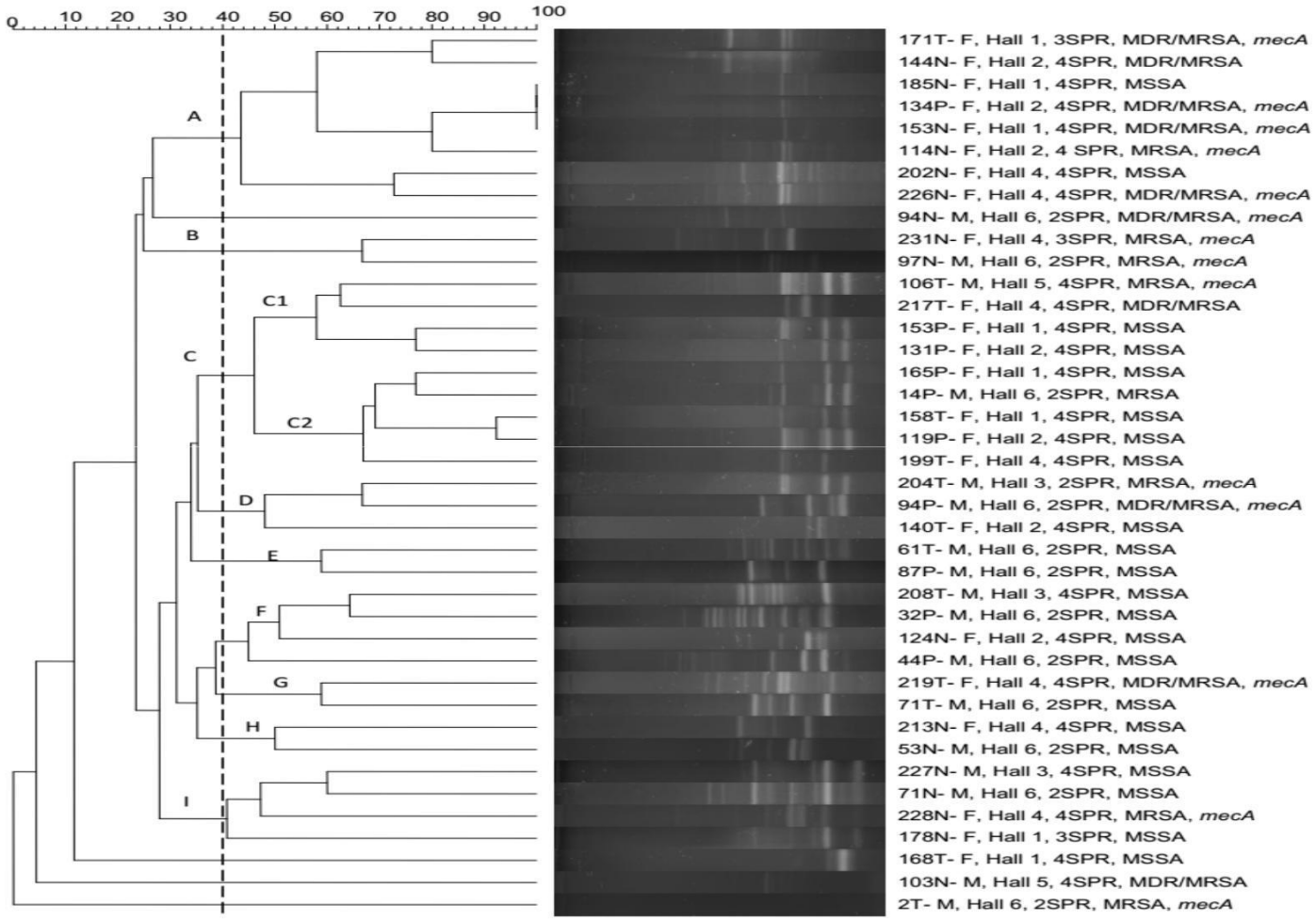
Type/source of isolate	Number of isolates tested	***lukFS-PV*** gene, n (%)
Overall	54	17 (31.5%)
MRSA	26	7 (26.9%)
MSSA	28	10 (35.7%)
MDR	16	4 (25%)
Nostril	22	7 (31.8%)
Fomites	32	10 (31.3%)

**MRSA, methicillin-resistant S. aureus= MDR, multi-drug resistant= MSSA, methicillin-susceptible S. aureus= Overall, all isolates that were selected to screen for the presence of the* lukFS-PV *gene*

Genetic relationships of *S. aureus* isolates recovered from the student population

A total of 40 isolates with different resistance phenotypes obtained from participants with different clinical and socio-demographic backgrounds were selected and analysed for genetic relatedness using the Repetitive element Sequence-Based PCR. At a 40% level of similarity, the 40 isolates fit into 10 different clusters identified as Cluster A to I (Figure 3). Cluster A isolates were all from female students. About 62.5% of the isolates in this cluster were MRSA that were also MDR. All the Isolates in cluster C were recovered from fomites. These isolates fit into two sub-clusters (C1 and C2). All isolates in sub-cluster C1 were recovered from participants who stayed in fours. All isolates in sub-cluster C2 (55.6%) were recovered from students who did not take part in sports and were also negative for *mecA* gene, although one of them was an MRSA. At least 90.9% of isolates in clusters D to G were recovered from fomites used by males mostly (72.7%) than females (27.3%). These isolates were found to circulate within halls two, three and six. All the isolates in clusters H and I were recovered from nostrils of both males and females in equal proportions. These isolates were also MSSA strains and were circulating with halls one, two, three, four and six.

Figure 3
Rep-PCR-generated dendogram for selected *S. aureus* isolates



*F, female= M, male= SPR, number of students per room= MDR/MRSA, Multi-drug resistant/methicillin-resistant *S. aureus*= MSSA, methicillin-susceptible *S. aureus.*

DISCUSSION

The approximate rate of *S. aureus* colonization among students in institutions of higher learning has been varied with prevalences below 25% being reported for nasal colonization in China and Nepal (12,13). In this study, we found prevalences above 25% for both nasal and fomites colonization. We found no reports highlighting the prevalences of colonization of fomites such as phones and pens, specifically those used by students in university settings in Kenya.

Consistent with the findings by Hogan et al. (14), the female gender was a risk factor for nasal colonization. Significantly high carriage of *S. aureus* and MRSA on nostrils and phones were found in several halls where students stayed in congested rooms of at least 4 occupants per room numbers. Possibly, this type of accommodation also facilitated sharing items such as phones, pens and other personal effects. Turabelidze et al. (15) reported that sharing such objects is a risk factor for the colonization by *S. aureus* and related MRSA strains. Kejela et al. (3) also reported congestion or overcrowding to be a possible risk factor for colonization by MRSA strains among student populations.

We found high resistance to ampicillin among the isolates in this study. Okamo et al.

(16) and Kejela et al. (3) also reported high ampicillin resistance among MRSA isolates recovered from a healthy student populations. All the isolates were sensitive to nitrofurantoin and linezolid, a finding that confirmed results reported by Kitti et al. (17) and Eko et al. (18). These findings could possibly suggest that nitrofurantoin and linezolid may be used effectively in the management of *S. aureus* related infections, a recommendation that was given by Watkins et al. (19). Resistances to chloramphenicol, erythromycin, gentamicin, ciprofloxacin, norfloxacin trimethoprim-sulfamethoxazole have been variable across different studies (14,18,20), an observation that prompts the necessity of continued screening of *S. aureus* isolates to determine the efficacy of the antimicrobials prior to their use in managing

S. aureus-related infections.

Our results established the existence of a relatively low prevalence of MRSA among a healthy population of university students but other studies have reported higher MRSA prevalence (18.7%) among student populations in Jeddah, Saudi Arabia (21). Other studies in Tanzania (16) and (17) have reported very low prevalences of 0.3% and 1% respectively among student populations. Hence, considering the case of this study where significantly high chances of MRSA colonization were reported in particular residence halls, it could be possible that other students visiting these residence halls would be increasing their chances of MRSA colonization.

Results from this study showed that SCC*mec*II and V were predominant among the isolates from the healthy student population. In Kenya, the SCC*mec*II, III, IV have been found to be most predominant is a pool of isolates recovered from health care settings in different studies (11,22,23). We did not find studies that reported the prevalence of these elements among isolates recovered from healthy populations. The predominance of SCC*mec*II could be attributed to their small size, and therefore, can be transmitted with ease within community settings (23). Due to scarcity of information on the prevalence of SCCmec elements within community settings, more studies need to be done in order to ascertain why certain elements are predominant within specific populations in Kenya. We did not find a statistically

significant association between carriage of the *lukFS-PV* and MRSA or MSSA strains. However, other studies have reported statistically significant increase in the prevalence of *pvl*-positive MRSA strains from clinical settings in regions such as Ireland and Germany (24–26). A study by Schaumburg et al. (27) reported a high prevalence (55.9%) of isolates from a health population carrying the *lukFS-PV* gene, but the reason and impact of this was unclear. We found a prevalence of 31.5%, which suggested a likelihood of existence of virulent strains among healthy populations.

Analysis of genetic relations of the isolates indicated a likelihood of strain sharing among students. This would be expected since the isolates were recovered from students who stayed in close proximities, and shared their personal items.

CONCLUSION

This study offers insights into the prevalence of carriage of *S. aureus* and associated MRSA strains in the nasal cavities, phones and pens of students in a university setting in Central Kenya. Among the factors found to be associated with increased transmission rates of *S. aureus*, was congestion in some hostel rooms (staying in fours). Based on this information, we find it important to allow students to stay in fewer numbers. Since we found no studies, which reported rates of colonization in students residing in different hostels within a single institution of higher learning, similar studies could help identify the public health significance of reducing the number of students sharing a hostel room to less than four. There should also be comprehensive surveillance studies on MRSA and control measures to help in curbing the spread of MRSA strains.

COMPETING INTERESTS

The authors declare that they have no competing interests

ACKNOWLEDGEMENT

This work was supported by the Centre for Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI) and the Environmental & Social Ecology of Human Infectious Diseases Initiative (ESEI), Grant Reference: G1100783/1"

REFERENCES

1. DeLeo FR, Otto M, Kreiswirth BNB, Chambers HFH. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet* [Internet]. 2010;375(9725):1557–68. Available from: http://www.sciencedirect.com/science/article/pii/S0140673609619991%5Cnhttp://dx.doi.org/10.1 016/S0140-6736(09)61999-1
2. Brown AF, Leech JM, Rogers TR, McLoughlin RM. *Staphylococcus aureus* colonization: Modulation of host immune response and impact on human vaccine design. *Front Immunol.* 2014;4(JAN):1–20.
3. Kejela T, Bacha K. Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. *Ann Clin Microbiol Antimicrob* [Internet]. 2013;12(1):11. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L52619231%5 Cnhttp://www.ann-clinmicrob.com/content/12/1/11%5Cnhttp://dx.doi.org/10.1186/1476-0711-12-11%5Cnhttp://sfx.hul.harvard.edu/sfx_local?sid=EMBASE&issn=14760711&id=doi:10.118
4. Walia SS, Manchanda A, Narang RS, Anup N, Singh B, Kahlon SS. Cellular telephone as reservoir of bacterial contamination: Myth or fact. *J Clin Diagnostic Res.* 2014;8(1):50–3.
5. Arulmozhi V, Janagond AB, Savitha S, Kalyani J, Sumathi G. Cell phones of healthcare providers as fomites harbouring potential pathogenic microogranisms. *Int J Curr Res Rev* [Internet]. 2014;6(19):1–4. Available from:

http://www.scopemed.org/fulltextpdf.php?mno=170604
6. Otto M. Improved understanding of factors driving methicillin-resistant *Staphylococcus aureus* epidemic waves. *Clin Epidemiol* [Internet]. 2013;5:205. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3707418&tool=pmcentrez&rendertype=abstract
7. CDC. Antibiotic Resistance Threats. *Cdc*. 2013;22–50.
8. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P & T: A peer-reviewed J Formul Manag* [Internet]. 2015;40(4):277–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25859123%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4378521%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25859123%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4378521
9. Mozzillo KL, Ortiz N, Miller LG. Patients with Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection – 21st Century Lepers. *NIH Public Access*. 2010;75(2):132–4.
10. Braga EDV, Aguiar-Alves F, de Freitas M de FN, de e Silva MO, Correa TV, Snyder RE, et al. High prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* colonization among healthy children attending public daycare centers in informal settlements in a large urban center in Brazil. *BMC Infect* Dis [Internet]. 2014;14:538. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4287590&tool=pmcentrez&rendertype=abstract
11. Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya. *Int J Infect Dis* [Internet].2013;17(2):e115–9. Available from: http://dx.doi.org/10.1016/j.ijid.2012.09.006
12. Du J, Chen C, Ding B, Tu J, Qin Z, Parsons C, et al. Molecular characterization and antimicrobial susceptibility of nasal *Staphylococcus aureus* isolates from a chinese medical college campus. *PLoS One*. 2011;6(11).
13. Ansari S, Gautam R, Shrestha S, Ansari SR, Subedi SN, Chhetri MR. Risk factors assessment for nasal colonization of *Staphylococcus aureus* and its methicillin resistant strains among pre-clinical medical students of Nepal. *BMC Res Notes* [Internet]. 2016;9(1):214. Available from: http://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-016-2021-7
14. Hogan B, Rakotozandrindrainy R, Al-emran H, Dekker D, Hahn A, Jaeger A, et al. Prevalence of nasal colonisation by resistant *Staphylococcus aureus* among healthcare workers and students in Madagascar. *BMC Infect Dis* [Internet]. 2016;1–9. Available from: http://dx.doi.org/10.1186/s12879-016-1733-6
15. Turabelidze G, Lin M, Wolkoff B, Dodson D, Gladbach S, Zhu BP. Personal hygiene and methicillinresistant *Staphylococcus aureus* infection. *Emerg Infect Dis.* 2006;12(3):422–7.
16. Okamo B, Moremi N, Seni J, Mirambo MM, Kidenya BR, Mshana SE. Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* nasal carriage among pre-clinical and clinical medical students in a Tanzanian University. *BMC Res Notes* [Internet]. 2016;9(1):47. Available from: http://www.biomedcentral.com/1756-0500/9/47

17. Kitti T, Boonyonying K, Sitthisak S. Prevalence of methicillin-resistant *Staphylococcus aureus* among university students in Thailand. *Southeast Asian J Trop Med Public Health.* 2011;42(6):1498–504.
18. Eko KE, Forshey BM, Carrel M, Schweizer ML, Perencevich EN, Smith TC. Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization and infection isolates in a Veterans Affairs hospital. *AntimicrobResistInfectControl*. 2015;4(2047–2994 (Electronic)):10.
19. Watkins RR, Lemonovich TL, File TM. An evidence-based review of linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): Place in therapy. *Core Evid.* 2012;7:131–43.
20. Ayepola OO, Olasupo NA, Egwari LO, Becker K, Schaumburg F. Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from clinical infection and asymptomatic carriers in Southwest Nigeria. *PLoS One.* 2015;10(9):4–11.
21. Zakai SA. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among medical students in Jeddah, Saudi Arabia. *Saudi Med J.* 2015;36(7):807–12.
22. Aiken AM, Mutuku IM, Sabat AJ, Akkerboom V, Mwangi J, Scott JAG, et al. Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. *Antimicrob Resist Infect Control* [Internet]. 2014;3:22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4107749&tool=pmcentrez&rendertype=abstract
23. Omuse G, Van Zyl KN, Hoek K, Abdulgader S, Kariuki S, Whitelaw A, et al. Molecular characterization of *Staphylococcus aureus* isolates from various healthcare institutions in Nairobi, Kenya: a cross sectional study. *Ann Clin Microbiol Antimicrob* [Internet]. 2016;15(1):51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27647271%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5029008
24. Melles, Damian C; van Leeuwen, Willem B; Boelens, Helene AM;Peeters, Justine K;Verbrugh H a; VB. Panton-Valentine Leukocidin Genes in *Staphylococcus* Small Anellovirus in Hepatitis C Patients and Healthy Controls. *Emerg Infect Dis.* 2006;12(7):1174–5.
25. Berktold M, Grif K, Mäser M, Witte W, Würzner R, Orth-Höller D. Genetic characterizationofPanton-Valentine leukocidin-producing methicillin-resistant *Staphylococcus aureus* in Western Austria. *Wien Klin Wochenschr.* 2012;124(19–20):709–15.
26. Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. aureus clones, and increasing multiresistance. *J Clin Microbiol.* 2014;52(3):859–70.
27. Schaumburg F, Kock R, Friedrich AW, Soulanoudjingar S, Ngoa UA, von Eiff C, et al. Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. *PLoS Negl Trop Dis.* 2011;5(5).