ORIGINS AND SPREAD OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN GREATER KABALE REGION - SOUTH WESTERN UGANDA

BY

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2014 /PhD/004/PS

THESIS SUBMITTED TO THE FACULTY OF MEDICINE IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY OF MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY

SEPTEMBER 2019

DECLARATION

I, Baguma Andrew do hereby declare that the work presented in this thesis is the result of my original research work. Where I have used the works of other persons, due acknowledgements are clearly stated. No portion of this work has been submitted in support of an application for a degree or qualification to any other university or institute of higher learning. I present it without any reservations for external examination.

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DEDICATION

Carrying out this research has been a joy and has given me a sense of deep satisfaction. It would not have been possible to devote such time and energy without the patience, understanding and dedicated love of all members of my family. The thesis is dedicated to them.

ACKNOWLEDGEMENT

This work could not have been possible without the financial assistance and moral support given to me by the German Academic Exchange (DAAD) and more especially, the Head of Microbiology department, Dr. Joel Bazira and the entire members of the department. I am sincerely grateful and may God put you in the school for a purpose for which I am grateful to Him.

My academic supervisor, Dr. Joel Bazira, who inspired me, encouraged me to carry on despite the odds, and above all read through the entire work, word by word on several occasions without getting tired, directing me till the end. You are my mentor. I pray never to disappoint you! I call Dr. Joel Bazira, my academic father!

During the initial stages of this study, I was guided and assisted in varying ways by Central Public health laboratories (CPHL) - Ministry of Health. The management provided me space and reagents to carry out primary laboratory work. Many thanks and honor goes to Ikoba Suleiman, Atek Kagirita and Steven Aisu for the support while I was doing laboratory work at CPHL – MoH. I thank these individuals for the assistance rendered to me.

I wish to extend my acknowledgement with gratitude for the support provided by Ass.Prof. Benon Asiimwe of Makerere University College of Health Sciences who sponsored detection of MecA gene by PCR free of charge for this study. Benon, I thank you immensely for the enthusiasm you gave to this study.

I wish to acknowledge Mr. Tonny Jimmy Owalla of Med Biotech who tirelessly kept providing me with a good molecular biology reagents and to which I was able to generate quality work I present today. Thank you.

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LIST OF ACRONYMS

µL	Microlitre
ANOVA	Analysis of variance
ATCC	American type culture collection
BD	Becton and Dickson
BURP	Based Upon Repeat Pattern
CA – MRSA	Community acquired Methicillin-Resistant *Staphylococcus aureus*
CC	Clonal complex
CDC	Centre for Disease Control and prevention
CLSI	Clinical and laboratory institute
HA – MRSA	Hospital acquired Methicillin-Resistant *Staphylococcus aureus*
IERC	Intuitional ethics and review committee
KRRH	Kabale regional referral hospital
LA – MRSA	Livestock associated Methicillin-Resistant *Staphylococcus aureus*
MOAIF	Ministry of agriculture animal industries and fisheries
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *staphylococcus aureus*
$Nacl_2$	Sodium chloride
PCR	Polymerase chain reaction
PVL	Panton valentine leukocidin
SCCmec	Staphylococcus aureus cassette chromosome
ST	Strain type
TSS	Toxic shock syndrome
UK	United Kingdom
HHC	Household contacts
OPD	Outpatient

IP	Inpatients
MGE	Mobile Genetic Elements
HCW	Healthcare workers
SpA	Staphylococcal protein A
MSA	Mannitol salt agar
CoNS	Coagulase negative staphylococcus
API	Analytical Profile Index
MALDI – TOF MS	Matrix assisted laser desorption ionization time of flight mass spectrometry
MLST	Multi Locus sequence typing
AFLP	Amplified fragment length polymorphism
MLVA	Multiple locus variable number tender repeat assay
RFLP	Restriction fragment length polymorphism
PFGE	Pulsed field gel electrophoresis
WGS	Whole genome sequencing
ID	Identification
AST	Antibiotic Susceptibility Testing
QC	Quality Control
ATM	Amie's Transport Media
ATCC	American Type Culture Collection
MHA	Mueller Hinton Agar
BAP	Blood Agar Plate
iCR	Inducible Clindamycin Resistance
HH	Household contact
OP HHS	Outpatient Household Swine
OPHHC	Outpatient Household Cattle
MEGA X	Molecular Evolutionary Genetic analysis
TMP-SMX	trimethoprim –sulfamethoxazole

ABSTRACT

Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) remains a major global healthcare problem responsible for increased motility, prolonged hospitalization and surging economic loss in the livestock. The prevalence in human across African continent is heterogeneous. The emergency of highly pathogenic clones is of solemn concern. We determined the nasopharyngeal carriage rate of MRSA, respective spa diversity and relatedness among human and livestock from greater Kabale region – south western Uganda.

Materials and methods: This was a cross sectional study, carried out in greater Kabale region, between June 2016 and April, 2019. Nasopharyngeal samples from 580 outpatients and 450 routine clinical samples were processed for identification of MRSA basing on inhibition zone diameter of ≤19 mm on 30 µg cefoxitin disk and MecA PCR. The positive MRSA outpatients were followed back to their respective house hold contacts (n =1536) and domestic cattle (n = 710) and swine (n = 585) swabbed and their nasopharyngeal swabs also analysed for presence of MRSA. The spa typing was performed using PCR – High resolution melting based method. The spa sequences analysed using MEGA – X software to determine phylogenetic relationship.

Results: A total of 487 MRSA isolates (18.9%) were recovered from human (OPD, HHC and IP) while 342 MRSA isolates (26.4%) were recovered from cattle and swine. A total 27 spa types were recovered among all isolates which included Spa types; t1081, t701, t034, t041, t044, t437, t037, t4677, t127 t1451, t9377, t032, t121, t1544, t922, t019, t108, t002, t030, t018, t202, t040, t021, t012, t1358, t267 and t325 were detected and the most frequent were t034(12.2%), t1081(8.9%), t701(8.9%), t4677(8.5%) and t1451 (6.9%). Spa types; t021, t012, t1358 and t325 were only recovered from inpatients.

Conclusion: The MRSA carriage rate in both human and animal of greater Kabale region is high with increasing spa types diversity. The origin of spread remains unknown but there are ongoing bidirectional transmissions between humans and animals. Infection prevention and control procedures in both community, human health-care settings and animal husbandry is urgently needed to restrict infections using one health approached.

Key word: **MRSA, Diversity and relationship**

CHAPTER ONE

INTRODUCTION AND BACKGROUND

1.1 Introduction

This is a report on the study undertaken during 2014 and 2018 into the origins and spread of Methicillin Resistant *S. aureus* (MRSA) in greater Kabale region – South Western Uganda. The findings provide a background information for policymakers, Public Health practitioners, Veterinary/Animal health practitioners, Health care providers and other stakeholders in Health on dynamics of MRSA and its resistance to other antibiotics that are commonly used in managing bacterial infections in both medical and veterinary settings in and round greater Kabale region. This knowledge provides insightful and awareness on the opportunities for prevention and control using one health platform.

1.2 Background

Staphylococcus is a member of the family Micrococcaceae. They are Gram positive bacterial cells in spherical shape of about 1μm in diameter. Microscopically, the staphylococci cells arrange in grape-like clusters but sometimes as single or paired. Over thirty species exists in the Staphylococcus genus (**Whitman *et al.*, 2012**). Some species exist as normal flora on skin and mucous membranes of human and other cold blooded animals, while other members can cause serious infection. Those of clinical importance are Staphylococcus aureus (S. aureus), Staphylococcus epidermidis and Staphylococcus saprophyticus. S. aureus is the commonest pathogens in humans worldwide (**Christiane *et al.*, 2015**).

DNA-ribosomal RNA (rRNA) hybridization and comparative oligonucleotide examination of 16S rRNA reveals that Staphylococcus is a group of bacteria occurring within the comprehensive family of Bacillus-Lactobacillus-Streptococcus cluster that are significantly Gram-positive bacteria with lower G + C content of DNA (**Ko, 2008**). Through DNA – DNA hybridization, several staphylococcus species have been identified belonging to taxonomic

Micrococcaceae family, among which a few can be isolated from humans as commensals for instance, *S aureus* (nares, axilla and perineum) and *S. epidermidis* (nares, skin) are common commensals and also have the greatest pathogenic potential. *S. saprophyticus, an* occasional skin flora is also among the common cause of urinary tract infection (UTI). *S haemolyticus, S simulans, S cohnii, S warneri* and *S lugdunensis* can also cause infections in man **(Anita *et al.*, 2019)**.

S. aureus was first described by Alexander Ogstonin 1880 from abscesses and wound infections and is common in nature and part of human normal flora (**Wolfgang *et al.*, 2009**). It is among the bacteria that are most resistant to the external environment and disinfectant agents especially while in microbial-derived sessile community known as biofilm which is typified by cells that are attached to a substratum, interface, or to each other, embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression and protein production (**Archer *et al.*, 2011**). *S. aureus* can cause various clinical situations varying between superficial infections and serious life-threatening infections like severe sepsis, pneumonia, and toxic shock syndrome (TSS). These conditions are facilitated by its propensity to secreted numerous virulence factors including a number of membrane damaging toxins capable of forming pores in the cytoplasmic membrane of host cells leading to cell lysis (**Stryjewski *et al.*, 2014**). This bizarre repertoire of virulence factors also allows it to survive extreme conditions within the human host and maintains fine control of virulence expression, and for the most part rarely causes severe infection in healthy individuals **(Liu, 2010)**. Bi-component leucocidins form an octameric structure of alternating S and F subunits that form a β-barrel pore spanning the lipid bi-layer of the host cytoplasmic membrane resulting in host cell lysis (**Vazquez, 2010**). Other potential virulence factors includes surface proteins that promote colonization of host tissues, invasins (kinases, hyaluronidase) that promote bacterial spread in tissues, surface factors (capsule, Protein A) that inhibit phagocytic engulfment, biochemical properties (carotenoids, catalase production)

that enhance their survival in phagocytes; immunological disguises (Protein A, coagulase) (**Todar, 2005**); exotoxins (SEA-G, TSST, ET) that damage host tissues or otherwise provoke symptoms of disease (**Souza *et al.*, 2014**). In addition, S. *aureus* capacity to form biofilms on host tissues and implanted medical devices, a major virulence traits underlying persistent and chronic infections. Biofilm is known to be part of ordinary life cycle of *S. aureus* in the environment, in which existing planktonic cells attach to solid surfaces, multiplying and accruing in multilayer cell clusters implanted in an organic polymeric matrix. The structural components of the biofilm matrix are mainly made up of proteins and polysaccharides, which allows accumulation of biomass, cohesion and stabilization. The existence of extracellular DNA into the biofilm matrix can promote genetic transfer horizontally with consequent AMR spread between embedded cells. Biofilm shelters and shields the bacterial cells from environmental stresses, host immune system, and antimicrobial destruction (**Plata, et al., 2009**). Biofilm thickness can range from a single cell layer to a substantial community encased by a viscous polymeric milieu. Biofilm confers the ability to evade multiple clearance mechanisms produced by host and antimicrobial due to dormant nature of phenotypes within (**Liu, 2010**).

In addition, one of the extra ordinary virulent factor is the pore-forming toxin known as Panton Valentine Leukocidin (PVL) toxin, produce by some strains mediated by pvl gene (**Ruban *et al.*, 2017**). This toxin causes life-threatening necrotizing infections, which are characterized by massive tissue inflammation and necrosis (**Stryjewski et al., 2014**). Therefore, *S. aureus* has evolved a comprehensive strategy to deal with the challenges posed by the immune systems of humans and animals qualifying as an indiscriminate life - threatening bacterial pathogen.

Since the introduction of antimicrobials, *S. aureus* bacteria have developed mechanisms for resisting the effects of antibiotics. The evolution and spread of *S. aureus* and other bacterial

species with increasing antimicrobial resistance stems from several factors, including widespread and inappropriate use of antibiotics, extensive use of these antibiotics as growth enhancers in animal feed, and intensification of regional and international travel **(Holmes Alison H. *et al.*, 2017)**. The occurrence and spread of multidrug resistance in Gram-positive bacteria is particularly a significant threat to current medical practice and perhaps *S. aureus* is the pathogen of greatest concern. Probably due its intrinsic virulence and ability to cause an assortment of severe disease conditions as well as its adoptability to different ecological conditions **(Brennan *et al.*, 2012).** The acquisition and inherent resistance to antimicrobial agents has aggravated the problem that has led to high morbidity and mortality by bacterial infection today **(Tenover, 2018)**. Of public health concern, the multidrug resistance *S. aureus* is now the leading cause of nosocomial infections and is becoming an increasing concern in the community setting. The mortality of *S. aureus* bacteremia remains approximately 20–40% and is now the leading overall cause of nosocomial infections and, as more patients are treated outside the hospital setting, is an increasing concern in the community **(Lowy, 2003)**.

The emergence and spread of multidrug resistant *S. aureus* represent a major public health threat. This phenomenon result from the convergence of a variety of factors that include mutations in common resistance genes that extend their spectrum of activity **(Cuny *et al.*, 2015)**, the exchange of genetic information with other microorganisms **(Wong *et al.*, 2015)**, the evolution of selective pressures in hospitals **(Vossenkuhl, 2015)**, livestock feeds **(Chang *et al.*, 2015)** and communities that facilitate the development and spread of resistant strains.

MRSA is among the highly resistant strains to multiple antibiotics limiting the choice of drug to manage infections **(Rossolini *et al.*, 2014)**. MRSA is resistant not only to the antibiotic methicillin, but also to wide variety of other antibiotics including vancomycin, making it very difficult to treat and, occasionally, deadly. This is the public health challenge especially in resource constrained settings.

Infections due to MRSA that originate in the community are an emerging problem in many parts of the world and are called community-associated MRSA (CA-MRSA). MRSA infections occurs in hospitals, in the community (**Lévesque *et al.*, 2014**) and animals (**van Duijkeren *et al.*, 2014**) as HA – MRSA, CA – MRSA and LA – MRSA respectively. These strains are indistinguishable phenotypically and can cross infect between animals and humans directly or indirectly (**Ballhausen *et al.*, 2014**). However, the prevalence, antimicrobial resistance profile especially towards the commonly used antibiotics, and the link between LA – MRSA, CA MRSA and HA – MRSA remains a mystery that requires to be elucidated among communities of Kabale region. Despite the fact that MRSA in Uganda has been described, it is limited to urban healthcare settings like Mulago National Referral Hospital (**Kateete *et al.*, 2014**) and data in other rural setting remains a challenge in the country.

1.3 Problem statement

Antimicrobial resistance has appeared to be a major threat to quality of healthcare leading to questionable patient safety. The rapidly dwindling antimicrobial armamentarium has consequently led to critical public health threat. Antimicrobial stewardship designed to improve antimicrobial usage in every healthcare settings has not helped much. Yet, it is supposed to provide good practice to achieve the finest clinical outcomes with minimal antimicrobial selective pressures which contributes to the emergence of resistance. There is urgent need for antimicrobial stewardship among Medical and veterinary practitioner to prudently act responsibly across respective institutional continuum of health care. However, the deficiency of knowledge on the prevalence, antibiotic profile and transmission dynamics has hindered such initiative. Consequently, this has escalated the burden of antibiotic resistance resulting from antibiotic misuse and overuse in the community as well as medical and veterinary practitioners prescribing ineffective antibiotics and ineffective doses of antibiotics to their irrespective clientele. It is evident that both health care workers, community and patients need to be educated on responsible antibiotic use or otherwise we

face huge health threat of antibiotic resistance. It is unclear about the source of MRSA, however, the hospitals and the community as well domestic livestock could be the major sources and there is no data available to justify this in Kabale district.

1.4 Purpose of the study

The purpose of the study was to establish the origin and spread of MRSA in greater Kabale region - western Uganda. Contextually, the study was to reveal the genetic relationship of MRSA isolates from humans (inpatients, Outpatients, Health care workers and Household contacts in the community) and their respective domestic animals (Cattle and Swine).

1.5 Justification of the study

Colonization of *S. aureus* and MRSA to humans and their respective domestic animals can be the source for infection and vice versa. *S. aureus* and MRSA infections most responsible for mortality and morbidity in both humans, Cattle and swine in Uganda. MRSA can easily be spread from human to human, human to animals and vice versa leading to frequent epidemics that may cause loss of life and economic income. Hand hygiene and isolation measures can be preventive for MRSA and efforts has been expended for a long time toward preventing hospital-acquired MRSA infections, and effective infection control measures have been put into practice in many parts of developed countries. However, no data is available on the MRSA prevalence and antimicrobial resistance rates as well as phylogenetic relatedness among isolates from humans, Cattle and Swine. Therefore, this was necessary aiming at determining the prevalence and antibiotic resistance rates of MRSA isolated from outpatients, inpatients attending KRRH and Health care workers and community household contacts as well as their respective domestic swine and cattle as well as phylogenetic relatedness of strains from different hosts evaluating strain transmission, elucidating the origin. This will be beneficial in building up essential component of an effective surveillance system to describe epidemiological trends and infection control strategies through distinguishing related or clonal isolates from unrelated isolates.

1.6 Research questions

1.6.1. What is the outpatient (OPD) nasopharyngeal carriage rate of MRSA - Kabale Regional Referral Hospital (KRRH)?

1.6.2. What is the household (HHC) nasopharyngeal carriage rate of MRSA in greater Kabale region?

1.6.3. What is the prevalence of MRSA among inpatients admitted at KRRH?

1.6.4. What is the prevalence of MRSA in livestock (cattle and swine) in greater Kabale region?

1.6.5. Are there differences among MRSA strains isolated from Domestic animals, HHC and OPD?

1.7 Objectives of the study

1.7.1 Major objective

To determine the origins and spread of MRSA in greater Kabale region - South Western Uganda.

1.7.2 Specific objectives

1.7.2.1 To determine the nasopharyngeal carriage rate of MRSA among the OPDs at Kabale Regional Referral Hospital (KRRH)

1.7.2.2 To determine the prevalence of nasopharyngeal carriage of MRSA among household contacts (HHC) in Kabale region

1.7.2.3 To determine the prevalence of MRSA among inpatients (IP) admitted at KRRH

1.7.2.4 To establish the prevalence of MRSA among cattle and swine in greater Kabale region

1.7.2.5 To establish the relatedness of MRSA strains isolated from IPs, OPDs and respective HHCs and their respective animals (swine and cattle) in greater Kabale region

CHAPTER TWO: LITERATURE REVIEW

2.1.Introduction

S. aureus is a human pathogen with considerable ecological ability to adapt new environment. It is a Gram positive bacterium usually known to establish themselves on the skin and in the nose of healthy individuals and forms an important opportunistic pathogen which persistently colonizes about 20% of the human population (**Foster *et al.*,2014**). It is the most important cause of human bacterial infections ranging from minor infections such as pimples (**Birnie *et al.*, 2008**) to serious and fatal infections like pneumonia (**Rubinstein *et al.*, 2008**). Approximately 20% to 30% of the human population continuous carriers of *S. aureus* as part of part of the normal flora of the skin flora, Nostrils, axillae, Perineum and lower reproductive tract of women. Even though *S. aureus* can be existing as normal flora on the skin of the host, a great percentage of its carriage is through the anterior nares of the nasal passages (**Todar, 2005**). *S. aureus* has co-evolution with its human hosts overtime, consequently adapting nasopharynx carriage without causing symptoms or infection. This has allowed continuous spreading throughout human population as its fitness increasing. There are approximately 50% of the human carriers of *S. aureus*, with 20% continuous carriers and 30% as intermittent. Several factors are believed to be determinants of *S. aureus* carried is asymptomatically in human host. These factors may possibly include age, sex, diabetes, smoking and genetic factor like polymorphism in the glucocorticoid receptor gene that results in larger corticoid production (**Mahon et al., 2015**).

2.2. Mechanism of Antibiotic resistance in *S. aureus* and MRSA

S. aureus has an exceptional capability of acquiring resistance to antibiotics (**Edwards *et al.*, 2013**). It has developed resistance to antimicrobials by diverse mechanisms, including regulating uptake of the drug, alteration of the drug target, enzymatic inactivation of the antibiotics, and active efflux mechanism. The bacteria may use one or more of these resistance mechanisms depending on the type of antimicrobial agent present. The resistance

genes are transferable genetic elements, like plasmids and transposons which facilitates horizontal transfer of resistance between *S. aureus* cells bacteria (**Deurenberg *et al.*, 2007**). *S. aureus* demonstrates a better adaptive evolution in the antibiotic period, as it has proved to have a unique capacity to rapidly respond to any new antibiotic with an improved resistance mechanism. Antibiotic resistances have been witnessed earlier against penicillin and methicillin, until most recent, linezolid and daptomycin (**Edwards *et al.*, 2013**).

Resistance mechanisms that includes enzymatic inactivation of the antibiotic e.g. penicillinase and aminoglycoside-modification enzymes empowers the *S. aureus* cells to survive in presence of penicillin and Aminoglycosides (Gentamycin) respectively (**Mwambi et al., 2014**).

Alteration of the target sites meant for antibiotic to initiate its action results into *S. aureus* cell to decrease affinity for the antibiotic. Notably, penicillin-binding protein 2a of methicillin-resistant S. aureus and D-Ala-D-Lac of peptidoglycan precursors of vancomycin-resistant strains has been common among *S. aureus* specie. Other antibiotics have been viciously trapped (vancomycin and daptomycin) without any serious effect on the cell **(Stefani *et al.*, 2012)**.

Some strains of S. aureus have developed an efflux pumps mechanism that pump out antibiotics like fluoroquinolones and tetracycline and consequently surviving in any concentration levels of the agent. Complex genetic arrays known as staphylococcal chromosomal cassette mec elements or the vanA operon have been acquired and established by *S. aureus* through horizontal genetic transfer **(Everitt *et al.*, 2014)**. Worrisomely, some strains of S. aureus have spontaneously mutated or acquired positive selection to resist against several antibiotics, including the most recent ones like fluoroquinolones, linezolid and daptomycin (**Lindsay *et al.*, 2014**).

Generally, the era of antibiotics is under threat in the world of *S. aureus* due to emergence of superbugs known as MRSA representing one of the public health threat against antimicrobial realm. MRSA strains has staphylococcal cassette chromosome *mec* (SCC*mec)* element harboring the *mecA* gene that codes for an alternative penicillin binding protein, PBP2a, which confers resistance to beta-lactams, the antibiotic class of first choice for treating *S. aureus* infections (**Kilic *et al.*, 2006**). An added threat is the emergence of vancomycin-resistant *S. aureus* (VRSA). The MRSA strains, may carry SCCmec, which carries the mecA gene and other genetic elements for other antimicrobials such as aminoglycosides (gentamicin, tobramycin) macrolides (erythromycin), and tetracycline. This confers the strains of MRSA, especially the hospital-acquired, ability to resist multiple antimicrobial agents (**Mwambi et al., 2014**).

Methicillin resistance is escalating, a trend that is causing serious threat to both medical providers and the public (**Kejela *et al.*, 2013**) as it is responsible for many difficult-to-treat infections leading to increased mortality and morbidity (**Brennan *et al.*, 2012**). More to that, Infections with MRSA have severely impaired treatment outcomes and are cost intensive for the healthcare systems (**Gomez *et al.*, 2014**). MRSA has adapted rapidly to the selective pressure of antibiotics that resulted into its emergence and spread within the hospital/health care settings as HA – MRSA (**Deurenberg *et al.*, 2007**), in the community as CA-MRSA(Ogata *et al.*, 2014) and LA – MRSA which is highly prevalent in livestock animals (**Köck *et al.*, 2013**).

CA – MRSA is genetically divergent from HA - MRSA infections with different epidemiological characteristics, clinical spectrum (**Wang *et al.*, 2013**) and the frequency of infection and spread is increasingly alarming (**Stevenson *et al.*, 2014**) especially in children and elderly members of the community (**Ho *et al.*, 2013**). Livestock-associated *S. aureus* (LA-MRSA) has been mainly linked to the clonal complex (CC) 398 mainly associated with

animals as major reservoir. However, MRSA in livestock, LA - MRSA CC398 has no distinctive host specificity where it can colonize or infect various animals species and humans (**Cuny *et al.*, 2013**). Interaction between humans and animals has been evidently implicated in the transmission dynamics of LA - MRSA strains (**Köck *et al.*, 2013**). Domestic animals can be reservoir for new human-pathogenic *S. aureus* clones with the capacity for pandemic spread where food chain is among the ways in which infection can occur (**Spoor *et al.*, 2013**).

Globally the outbreak of MRSA infections is high and particularly highly reported in developed countries. In Africa continent, the prevalence appears to be heterogeneous with the much of the data coming from Mediterranean basin, South Africa, and Nigeria. However, the burden of MRSA in Africa is not negligible and ranges between 25–50% across the continent. The differences could be contributed by country variations in antibiotic accessibility and use. Moreover, HIV scourge and tuberculosis could be the probable cause for differences in MRSA prevalence among sub-Saharan countries (**Kesah *et al.*, 2003**). In Uganda, the limited data on MRSA prevalence is far beyond. There few studies that has reported on MRSA, moreover targeting specific locality or clinical malady. In some study, MRSA prevalence has been reported to be ~10% among *S. aureus* isolated from septic surgical sites of patients undergoing surgical treatment at Mulago National Hospital in Uganda in 1988. But the trends of MRSA prevalence in in the same hospital (**Kateete *et al.*, 2011**) and other hospital setting reporting alarmingly high results (**Baguma *et al.*, 2019**), (**Lubega, *et al.*, 2017**).

2.3.Taxonomy of *Staphylococcus aureus*

Taxonomically *S. aureus* belong to the genus Staphylococcus, in the family known as Staphylococcaceae. The same family includes other five less known genera like Gemella, Jeotgalicoccus, Macrococcus, Nosocomiicoccus and Salinicoccus. There are currently 47

recognized species of staphylococci and 21 subspecies, most of which are found only in lower mammals **(Prax, et al.,2019).** The staphylococci that are habitually associated with humanoid infections remains *S. aureus, S. epidermidis* and *S. saprophyticus*. However, other Staphylococcus species may also be associated with human infection **(Hogg, 2005)**. Phylogenetically, Staphylococcus is related to the members of the genus Bacillus in the family Bacillaceae and Listeriaceae **(Todar, 2005)(Whitman et al., 2012)**.

2.4.Community associated MRSA

CA-MRSA has emerged recently, and its origin and ability to spread in the community is still not clear. However, horizontal gene transfers of the resistance genes from coagulase-negative staphylococci (CoNS) to CA - MSSA is postulated. In fact, *S aureus* and CoNS may coexist on the skin of patient previously treated with beta-lactam antibiotics, giving a conducive environment for selection of CA-MRSA strains after horizontal transfer of resistance genes (**Sowash *et al.*, 2014**). CA-MRSA cells are genetically distinct from hospital strains as they grow more rapidly in vitro and carry few resistance genes with a novel mobile genetic element known as the arginine catabolic mobile element, which is also present in the skin commensal S. epidermidis. this may enhance fitness and pathogenicity of CA-MRSA placing it into highly competitious bacteria in the environment, unlike HA-MRSA which dominates in hospital settings (**Stryjewski *et al.*, 2008**). Therefore, CA-MRSA strains has unique genetic element that mediates methicillin resistance (the staphylococcal cassette chromosome mec type IV) and resistance to fewer antimicrobial drugs.

CA-MRSA strains characteristically carrying the Panton–Valentine leukocidin (PVL) genes, which produce cytotoxins that cause tissue necrosis and leukocyte destruction and are associated with staphylococci causing skin infections and necrotizing pneumonia (**Wood *et al.*, 2012**). CA-MRSA is a global problem contributing to a series of different lineages; and specific strains are predominant in different countries (**Sowash *et al.*, 2014**). They are more genetically diverse and belong to different clones. Some clonal complexes spread

internationally while others are restricted to certain regions (**Boswihi *et al.*, 2018**). Skin and soft tissue infections (SSTI) account for at least 90% of CA-MRSA infections. CA-MRSA SSTI are usually moderately severe to severe, often very painful, and are treated by simple incision and drainage. However, rare cases of very dramatic skin infections, such as necrotizing fasciitis, have been reported with CA- in contrast to HA-MRSA (**Stryjewski *et al.*, 2008**). CA-MRSA strains also cause infections of the bones and joints, such as osteomyelitis, and respiratory infections, such as pneumonia, sepsis, and urinary tract infections (**DeLeo *et al.*, 2010**)

Community-associated MRSA infections (CA-MRSA) are MRSA infections in healthy people who have not been hospitalized or had a medical procedure (such as dialysis or surgery) within the past year (**Dulon *et al.*, 2014**). CA-MRSA strains combine methicillin resistance with enhanced virulence and fitness (***Kong et al.*, 2016**). All CA-MRSA strains typically carry a novel type of methicillin resistance locus that appears to cause less burden but possession of specific toxin genes that encodes for Panton-Valentine leukocidin (PVL), alpha-toxin and phenol-soluble modulins (PSMs) (**Rasmussen *et al.*, 2012**). Acquisition of *speG* gene is some strains has enhanced virulence and versatility by polyamine tolerance, enhanced biofilm formation, adherence to fibrinogen/fibronectin, and resistance to antibiotic and keratinocyte-mediated killing (**Otto, 2012**). This gives some strains of CA – MRSA major selective advantage during skin infection and colonization, contributing to the extraordinary evolutionary success (**Watkins *et al.*, 2012**). The enhanced virulence of CA-MRSA strains is accompanied by enhanced survival in human neutrophils, suggesting that the interaction of the pathogen with neutrophils is a critical step (**Rigby *et al.*, 2012**). As evasion of neutrophil killing one of the predominant factors assumed to be associated with that enhanced virulence, staphylococcal leukocidin describes the molecular basis of CA-MRSA virulence (**Surewaard *et al.*, 2013**), (**Greenlee-Wacker *et al.*, 2017**). To be slightly detailed, Panton-Valentine leukocidin (PVL) is a two-component leukocidin that belongs to a

beta-barrel forming family of cytolytic toxins, also comprising several other leukocidins, gamma-toxin, and alpha-toxin(**van Duijkeren *et al.*, 2014**), (**Biber *et al.*, 2012**). PVL is encoded by the prophage-encoded adjacent lukS and lukF genes, which produce the two toxin parts, LukS and LukF (**Stegger *et al.*,2010**) and both are needed for the cytolytic activity of the toxin. **(Otto, 2013). (Maina et al., 2013), (C. Kim *et al.*, 2014), (HPSC, 2013).**

CA-MRSA strains are distinguished from HA-MRSA strains by harboring different types of SCCmec elements, the mobile genetic elements (MGEs) encoding methicillin resistance genes. Traditionally, they are usually sensitive to most antibiotics other than methicillin and beta-lactams in comparison to HA-MRSA isolates (**Reygaert, 2013**). However, occurrence of multi drug resistant CA-MRSA have been reported (**Márquez-Ortiz *et al.*, 2014**).The SCCmec elements found in CA-MRSA are types IV and V (**Milheiriço *et al.*, 2017**).

2.5.Nasopharyngeal carriage rate of MRSA

The nasopharynx is the upper part of the throat behind the nose while connecting the pharynx and comprises of three separate segments. the nasopharynx, oropharynx, and the hypopharynx (**Rocha *et al.*, 2017**). *S. aureus*, primarily exists as a commensal organism in a number of different anatomical sites including the nasopharynx. Nasopharyngeal bacterial colonization may be influenced by several factors, such as age, genetic background, geographic area, and environmental conditions (**Rocha *et al.*, 2017**). *S. aureus* is a common human pathogen that can colonize the respiratory tract and cause infection (**Van Nguyen *et al.*, 2014**). Although *S. aureus* can be present on the skin of the host, a large proportion of colonises the anterior nares of the Nasopharyngeal passages (**Wertheim *et al.*, 2005**). Usually spreads through direct physical contact or through contact with objects contaminated with infected bodily fluids and manifest as skin infections (**Witzel *et al.*, 2014**). It has been traditionally believed that the primary site of *S.aureus* colonization is the nasopharynx in

approximately 25% to 40% of the population **(Albrecht *et al.*, 2015)** and colonizes the anterior nares of 20–80% of the human population (**Brown *et al.*, 2013).**

The weakened or defective host immunity provides Nasopharyngeal passages defenceless against *S. aureus,* consequently evading host's innate immune system and uninterrupted colonisation **(Quinn et al., 2007).** There are high chances of colonization among the immunocompromised individuals **(Gebremedhn *et al.*, 2016)** and earliest days of human life (infancy) **(Patel *et al.*, 2015).** As earlier mentioned, nasopharyngeal carriage rate varies from place to place and age (**George *et al.*, 2016**) (**Witzel *et al.*, 2014**).

Although colonization is inoffensive, colonized individuals are at risk of endogenous infection when *S. aureus* enters otherwise sterile sites via wounds or indwelling medical devices **(Chessa *et al.*, 2016).** Colonization by *S. aureus* is a dynamic process which presents a substantial challenge that requires not only attaching to Nasopharyngeal epithelial cells, but also capacity to cope with existing host defenses and competing normal microbial flora. *S. aureus* attaches and invades host epithelial cells by means of various molecules that are collectively designated as "Microbial surface components recognizing adhesive matrix molecules" (MSCRAMM), overcoming host immune machineries and outcompeting existing resident microbial flora while secreting proteins and other factors such as wall teichoic acid (clumping factor B and wall-associated teichoic acid) coupled with constant replication to maintain its niche **(Albrecht *et al.*, 2015).** These innumerable interactions offer a strong advantage of *S. aureus* invasiveness over the hosts immune defense system **(Brown *et al.*, 2013).** In fact, the evolutionary implications of this may explain why *S. aureus* is such a capable pathogen since various proteins involved in colonization are essentially virulence factors **(Todar, 2005).** Colonization with methicillin-resistant *S. aureus* (MRSA) strains seems to confer a higher risk of subsequent invasion in hospitalized patients probably due to longer hospital stays and broader anti-microbial exposure **(Abi-Haidar *et al.*, 2011).** A study which matched patients for MRSA Nasopharyngeal carriage found that those with multiple

hospitalizations or a central venous catheter *in situ* were more likely to develop *S. aureus* bacteremia, which may explain the aforementioned findings **(Gupta *et al.*, 2013)**.

2.6.Nasopharyngeal carriage rate of MRSA among health care workers

Methicillin-resistant *S. aureus* (MRSA) is one of the most important hospital-associated (HA) pathogens. Health care workers (HCW) may become colonized with MRSA following contact with MRSA positive people in a healthcare facility or in the community **(King *et al.*,2016)**. HCW are most often transiently colonized, but they may become persistent carriers if they have chronic dermatitis or sinusitis, and this may lead to prolonged MRSA transmission **(Kumar *et al.*, 2013)**. It is likely that HCW can be important in the transmission of MRSA, but more frequently act as vectors, rather than being the main sources of MRSA where hand contamination is the most important mode of transmission **(Sassmannshausen *et al.*, 2016).** Transmission of MRSA from HCWs to patients has been reported describing MRSA outbreaks that have been epidemiologically linked to colonized or infected HCWs, especially those with exfoliative skin conditions, skin infections or respiratory tract infections **(InGram *et al.*, 2015)**. However, other studies have demonstrated that colonized HCWs are rarely the source of MRSA transmission to patients **(Dulon *et al.*, 2014)(Ćirković *et al.*, 2014)**.

Preventing transmission of drug-resistant organisms such as MRSA is a high priority for infection control and prevention. However, routine screening of asymptomatic HCWs for MRSA colonization is important since MRSA transmission, is more likely due to poor hand hygiene resulting in patient-to-patient transmission **(John *et al.*, 2015)**. Nasopharyngeal decolonization of HCWs with mupirocin has been recommended by several reports **(Thompson *et al.*, 2013)(Coates *et al.*, 2009)**. Critical questions about use of this antibacterial agent have been raised especially when to start the treatment **(Coates *et al.*, 2009)** as well as concerns of mupirocin resistant MRSA **(Agarwal, et al.,2015)**. Such

particular control measures has been tried with unsuccessful results in various hospital settings (**Gurieva *et al.*, 2012**).

2.7. MRSA in healthcare setting

HA – MRSA is a common pathogen worldwide and its multidrug resistance is a major concern. This strain is more in hospitals and other healthcare settings, with unique characteristic of multi drug resistance to several antibiotics (**Jayaweera *et al.*, 2017**) (**Bae *et al.*, 2004**). It continues to present life-threatening bloodstream infections, pneumonia and surgical site infections among hospitalized patients (**Gould *et al.*, 2012**). These comprise several MRSA Clonal complexes (CC) for instance CC5, CC8, CC22, CC30 and CC45 with CC5 and CC8 forming the most prevalent Clonal complexes worldwide (**Shambat *et al.*, 2012**). However, there is evidence of CC8-ST239-III-t037 lineage spreading from hospitals into the community which are now regarded as CA-MRSA (**Mediavilla *et al.*, 2012**). HA-MRSA clones have disseminated globally (**Harris *et al.*, 2010**). Infected and colonized human individuals constitute a major reservoir of *S. aureus*, and the primary route of transmission seems to be direct contact with infected or colonized individuals. In hospital facilities, large number of people, including patients, HCWs and visitors could be carriers of MRSA (**Ćirković *et al.*, 2008**). However, environmental spread may be significantly underestimated way in which transmission occurs in hospital (**Thompson *et al.*, 2013**). However, hospital staff can take some steps to prevent the spread of MRSA including; frequently washing hands between patient, washing hands after coming into contact with soiled linens or other items, wearing gloves when necessary and keeping hospital environment clean (**Whelan *et al.*, 2011**).

2.8.Livestock associated MRSA

In 2005, the first case of MRSA was reported in Netherlands among Swines (**Weese, 2010**). The epidemiology of MRSA in animals has gained interest because of increasing infectious processes and emergence of some clonal lineages associated with zoonotic potential (**Isa *et***

al., **2016)**. Since the first report of MRSA in cows in 1970, this bacteria has continued to spread causing serious economic loss **(Weese, 2010) (Vossenkuhl, 2015)**. Livestock associated - MRSA (LA – MRSA) infections have also been detected in farmers and some cases of MRSA of Clonal Complex 398 (CC398) have been identified in people without contact with animals **(Kigozi *et al.*, 2017)**. There are other clonal lineages (CC1, CC5, CC9, CC97, and CC130) of LA-MRSA that are emerging, and whose importance is increasing **(Lozano et al., 2016)**. There has been growing interest in studying *S. aureus* (MRSA and MSSA) in recent times. These strains play an essential role in the evolution of different genetic lineages **(Elhani *et al.*, 2015)**. MRSA prevalence and its respective antimicrobial resistance as well as its clonal lineages in humans, animals and food in Africa has great relevance, taking into consideration of high diversity in economic set up where the under resourced settings are hit hard ***(Khan et al.*, 2017)**. The close contact among humans, livestock, and domestic and wild animals, can have a relevant impact on the epidemiology of MSSA and MRSA**(Ammar *et al.*, 2016).** However, little has been done study the dynamics of this association. LA-MRSA strains can be found in humans especially those in close contact **(Garcia-Graells *et al.*, 2012).** Rates of colonization with LA- MRSA type 398 have been reported among humans **(George *et al.*, 2010)(Garipcin *et al.*,2015)**. However, the prevalence rates in the general human population is still not known. The emergence of LA - MRSA infections to humans is of public health concern. The strains belonged to clonal complex, CC398, with the majority of strains belonging to ST398. The infection with LA – MRSA could be related to horizontal transfer by means of wet hands of colonized or infected animal handler or farmers **(Schaumburg *et al.*, 2014)**. This transmissibility between bovine and human MRSA may cause severe infections **(Lindsay *et al.*, 2014)**.

LA-MRSA) are mainly associated with the clonal complex (CC) 398 and the main reservoir is the livestock, however this pathogen can also infect humans **(Chao *et al.*, 2014)**. Carriage of LA-MRSA may be common in people who have frequent contact with livestock

(**Graveland *et al.*, 2011**) which forms the most important risk factors followed by working with slaughterhouses (**van Duijkeren *et al.*, 2014**). Public health consequences of LA-MRSA infections pose a serious challenge and persons in direct contact with MRSA-positive animals have an increased risk of becoming MRSA positive (**Dryden, 2009**). MRSA CC398 is more often than not associated with asymptomatic colonization, several cases of infections can occur ranging from soft tissue infections (SSTIs) to endocarditis pneumonia and necrotizing fasciitis (**Dryden, 2009**). Risk factors for humans acquiring this LA-MRSA are Swine and cattle farming and frequently, it has been reported in different food production animals including cattle (**Faccioli-martins, 2011**). Airborne MRSA colonization have been documented in various studies (**Schulz *et al.*, 2012**).

LA-MRSA strains varies geographically where CC398 are predominant in European and American countries while ST39 is much more in Asian countries (**Crombé *et al.*, 2013**). Swine population are the main carriers of ST49 , LA-MRSA CC398, CA-MRSA CC30-V, and CC1 (**Cuny *et al.*, 2015a**) while in cattle LA-MRSA ST398have been largely observed (**Joshi et al., 2014**) Recently, CC130, ST425, and CC1943 group of LA-MRSA strains have been discovered in humans and dairy cattle, thought to be bovine-specific but now can infect humans (**Fluit, 2012**). There is continuous evolution of *S. aureus* strains in the animal and human settings. Fore stance, a new mec determinant, $mecA_{LGA251}$, which shares 70% homology with mecA, mong MRSA strains has been found in both cattle and humans. (**Sakwinska *et al.*, 2011**).

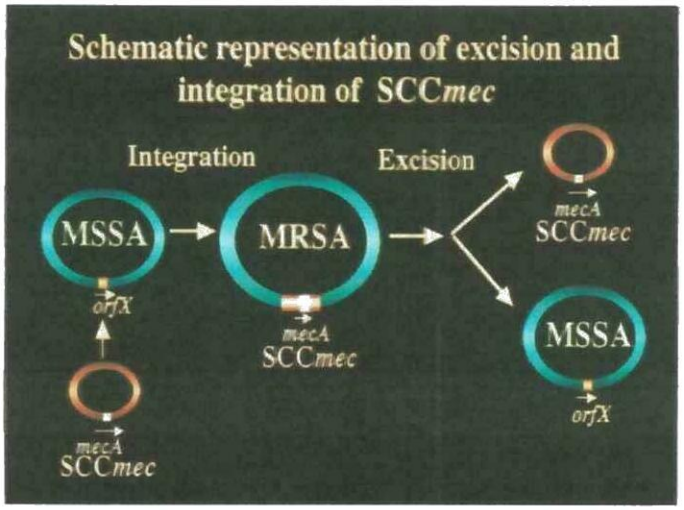
2.9. Genetics and phylogeny of MRSA

As depicted in the figure 01 according to Kuroda *et al*, *S. aureus* genome is approximately 2.81 Mbp in size with a relatively low G+C content (32.8%) with approximately 20 copies of insertion sequences and five transposons (***Kim et al.*, 2014**)(**Baba *et al.*, 2008**). Most regions of the genome well conserved with several large sequence blocks display high variability in genomic islands of which three are pathogenic islands. Genomic islands are likely acquired

horizontally with dedicated DNA recombination (integrase) genes **(Everitt *et al.*, 2014)**. This gives the capacity to thrive in the presence of penicillin-like antibiotics because of acquisition of extra chromosomal genetic elements containing resistance genes such as, plasmids, transposable genetic elements, and genomic islands **(Lindsay *et al.*, 2014)**.

S. aureus genomic sequences carry variable regions that frequently harbors virulent and antibiotic resistance genetic determinants that aid Staphylococcal bacterial invasiveness and development of diseases. Variable regions can be classified as prophages, pathogenicity islands, or staphylococcal cassette chromosomes **(Katayama *et al.*, 2000)**. Consequently, the overall combination these sequence elements and the encoded spectrum of virulence proteins vary from strain to strain and seems to be insightful of the general spectrum of clinical disease manifestations in humans **(Everitt *et al.*, 2014)**. The common resistant gene is *mecA* gene which is located within *Staphylococcal* cassette chromosome (SCC*mec*) genomic island **(Malachowa *et al.*, 2010)**. This is crucial in conferring *S.aureus* resistance to Methicillin and other beta lactam antibiotics **(Laabei *et al.*, 2014)** as well as other virulent factors **(Kaito *et al.*, 2011)**. The defining feature of MRSA is the SCC*mec*, and has been detailed on http://www.staphylococcus.net. This mobile genetic element carries the central determinant for broad-spectrum beta-lactam resistance encoded by the *mecA* gene **(Ito *et al.*, 2009).** *MecA* gene encodes penicillin-binding protein 2a (PBP2a), which differs from other penicillin-binding proteins and thus, does not bind Methicillin or other β-lactam antibiotics **(Harrison *et al.*, 2013)**. This results into the inability of PBP2a to interact with β-lactam moieties, thus conferring resistance to all β-lactam antibiotics **(Askari *et al.*, 2012)**. However, *S.aureus* has other thirty genes that has been well characterized as virulence genes responsible for pathogenesis **(Bae *et al.*, 2004)**. Many different genes (particularly antibiotic resistance genes) that seem to have been acquired horizontally and have driven the evolution of this superbug (**Kuroda et al., 2001**).

is traditionally perceived as the principal route of genetic transfer **(Fabio et al., 2017)**. There is little evidence that suggests transformation mechanism occurs in *S. aureus*. In addition, genetic changes due to transposon elements is not widespread among *S. aureus* **(Haaber *et al.*, 2017)**. Acquisition of SCC*mec* gene in Methicillin-sensitive *S. aureus* HW(*MSSA*) gives rise to a number of genetically different MRSA lineages which includes several MRSA clones: ST2470-MRSA-I, ST239-MRSA-III, ST5-MRSA-II, and ST5-MRSA-IV responsible for causing hospital-acquired MRSA (HA-MRSA) infections **(Wolters *et al.*, 2011)**. Different SCC*mec* genotypes give *S. aureus* different microbiological characteristics like antimicrobial resistance rates and differences in infectiousness. Types I-III SCC*mec* is routinely isolated from HA – MRSA strains, and types IV and V are associated with CA-MRSA strains **(Hudson *et al.*, 2013)** and CC398 is associated with livestock **(Bosch *et al.*, 2013)**. The staphylococcal cassette chromosome mec (SCCmec) elements are one of SCCs, widely dispersed in the genus staphylococci. SCCmec differs from other SCCs by the bearing the methicillin resistant-determinant genes. *S. aureus* susceptible to beta lactam antibiotics transforms to MRSA upon the acquisition of SCCmec. It is possible for MRSA to transform into MSSA upon losing the SCCmec gene(**Ito *et al.*, 2009**).



***(source* http://www.staphylococcus.net)**

Figure 02. Schematic representation of excision and integration of SCCmec

SCCmec elements carry a mec gene complex (mec) where the manifestation of *mecA* gene is controlled by two proteins, MecI (a repressor protein) and MecR1(signal transducer protein). The transcription of *mecA* is repressed through MecI binding to the promoter region of *mecA*. The binding of β-lactam antibiotics onto the MecR1 results into release of protease enzyme from the MecR1 to degrade MecI, causing increased transcription of *mecA*. Both *mecI* and *mecR1* genes are located upstream of *mecA*. The two genes are at times deleted or reduced by the integration of insertion sequences, IS*431* or IS*1272*. Insertion sequence IS*431* is situated downstream of *mecA* (**Katayama, et al., 2000**). Therefore, these gene alleles are *mec* gene complexes are composed of IS*431mec*, *mecA*, intact or truncated sets of regulatory genes, *mecRI* and *mec1*, or insertion sequences IS*431* or IS*1272*. Four classes of *mec* gene complex have been reported as follows as tabulated (Table 1)

Table1: Classes of mec gene complex

Class	*mec* gene complex
A	IS*431-mecA-mecR1-mecI*
B	IS*431-mecA*-Δ*mecR1*-IS*1272*
C	IS*431-mecA*-Δ*mecR1*-IS*431*
D	IS*431-mecA*-Δ*mecR1* *

**(no insertion sequences were identified so far tested by long-range PCR*

In addition, SCCmec elements also carry *ccr* gene complex which is composed of one or two site-specific recombinase genes, responsible for the mobility of SCC*mec*. The function of surrounding *orfs* is still unknown. The *ccr* genes are capable of catalysing excision and site-specific integration of SCC*mec* elements. There are four allotypes of *ccrA* and *B* genes known. The *ccrA1* and *ccrB1* are carried by type-I SCC*mec* while *ccrA2* and *ccrB2* is carried by type-II SCC*mec* and type-IV SCC*mec*. However, *ccA3* and *ccrB3* are carried by type-III *SCCmec* while *ccrA4* and *ccrB4* (carried by type-VI SCC*mec*)(**Shore *et al.*, 2013**). A *ccr* gene designated *ccrC* also exists in the type-V SCC*mec* element or SCC element e.g., SCC*mercury*. All ccr proteins (CcrA, CcrB, and CcrC) exists with catalytic motifs at the N-

terminal domain, a characteristic for recombinases of the invertase/resolvase family(**Shore et al., 2013**)(**Katayama et al., 2000**).

SCCmec elements have characteristic of directly-repeated nucleotide sequences (DRs) and inverted-complementary sequences (IRs) at both ends. They integrate into integration site sequence(ISS) for SCCmec which is located at 3' end of orfX. Characteristic nucleotides at both ends, direct repeats (DR) and inverted repeats (IR), are located at both ends.

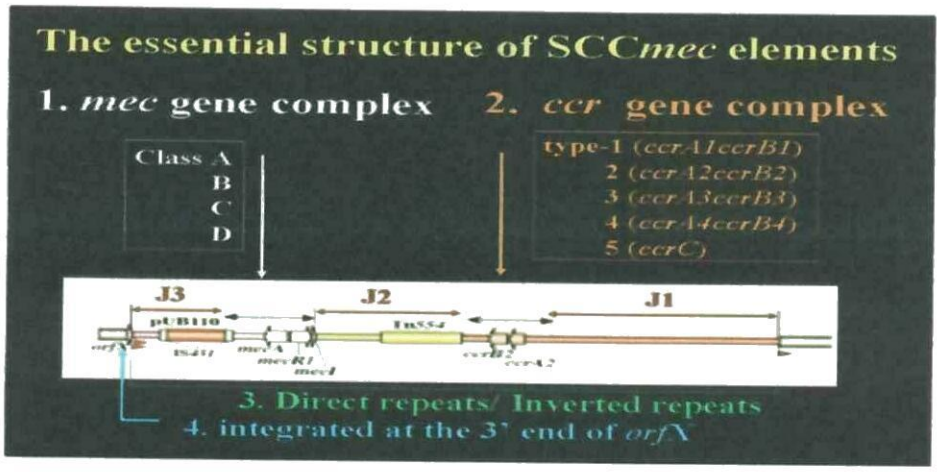


Figure 03. ***The SCCmec elements***

Note that the regions designated junkyard (J) regions (J1 (the region between ccr and the chromosomal region flanking SCCmec), J2 (the region between mec and ccr), and J3 (the region between orfX and mec).

Diversities in the structure of SCCmec elements are somewhat different. Allotypic variations can be found in both ccr and mec. Four classes of mec and five types of ccr have been reported as well as metamorphosis in J regions. Classification of SCCmec elements is currently based on the differences in mec, ccr and J regions as indicated below;

Type I	**type-1 ccr carrying ccrA1 and B1 and class B mec (1B)**
Type II	**type-2 ccr carrying ccrA2 and B2 and class A mec (2A)**
Type III	**type-3 ccr carrying ccrA3 and B3 and class A mec (3A)**
Type IV	**type-2 ccr carrying ccrA2 and B2 and class B mec (2B)**
Type V	**type-5 ccr carrying ccrC and class C mec (5C)**
Type VI	**type-4 ccr carrying ccrA4 and B4 and class B mec (4B)**

The C-terminal half of the protein is used to target the protein for attachment on the extracellular surface to the peptidoglycan cell wall via the LPXTG motif. The region X domains are designed to span the thick carbohydrate-rich covering on the bacterium. Thus, the N-terminal half extends past this covering and is available to bind to the host proteins **(Vazquez, 2010)**. The five antibody-binding domains in the N-terminal half SpA have high sequence identity (figure 02). The B domain of SpA and is a rapidly unfolding and folding (RUF) domain and the entire N-terminal half of protein A is a RUF domain protein **(Kobayashi et al., 2013)**.

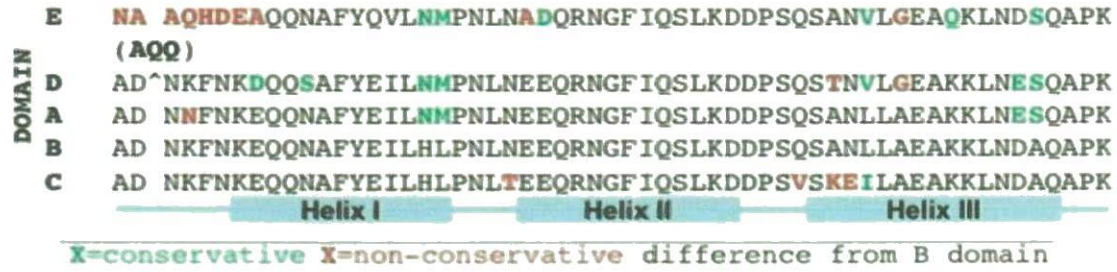


Figure 05. The antibody binding domain sequences of spa

Furthermore, SpA associates with the Fab portion of VH3-type IgM B cell receptors, mediating their cross-linking and leading to activation and clonal expansion of B lymphocytes and their subsequent apoptotic collapse (Figure 01).

The protein is anchored to the cytoplasmic membrane by a hydrophobic C-terminal domain **(Todar, 2005).** Region X, part of which comprises tandemly repeated octapeptide units, responsible for the molecule spanning the peptidoglycan of the cell wall **(Mayerhofer *et al.*, 2015)**. Protein A is a virulence factor and it impairs opsonophagocytosis of bacterial cells, induce hypersensitivity, complement activation and histamine release from basophils **(Foster *et al.*, 2014)**. Protein A is coded by spa gene, where the repeated part is located at 3'end and identified as X region; the repetitive part of region X consists of up to 12 units each with a length of 24 nucleotides. These 24-nucleotide region is highly polymorphic with respect to

the number and sequence of repeats leading to diversity of X region with consequent protein A variation (**Vossenkuhl, 2015**).

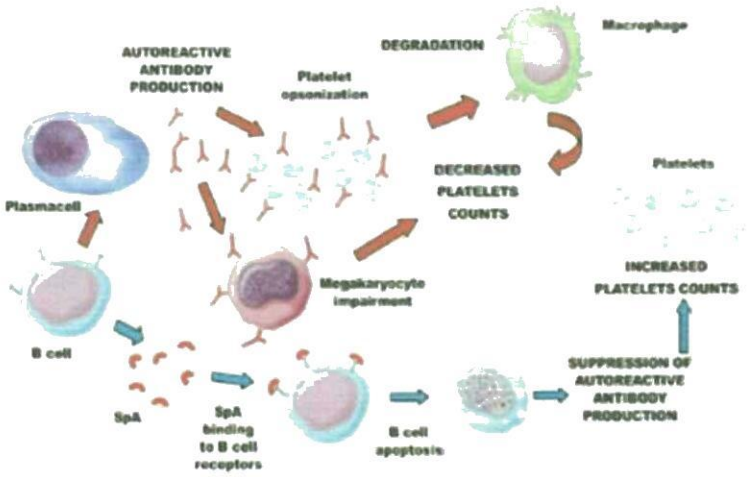


Figure 06. Pharmacological effects of spA binding to the Fab region of B cell receptors in ITP

The number of repeats in the region X of spa gene is related to the dissemination potential of MRSA, and associated with higher epidemic capability **(Omar *et al.*, 2014)**.The polymorphic X region in the *spa* gene is relatively stable and has discriminating power between the levels of PFGE and MLST **(Thomas *et al.*, 2009)(Michael *et al.*, 2013)**. *Spa* typing is easy to use with quick standardization, high discriminatory power, simplicity, reproducibility and portability and its combination with Based Upon Repeat Pattern (BURP) analysis becomes a useful tool for epidemiological typing and phylogenetic **(Sassmannshausen *et al.*, 2016)**. These methods can be useful for monitoring MRSA spread between hospitals, communities (OPD and Household contacts) and domestic animals **(Sakwinska et al., 2011)**.

In addition, High-resolution melting analysis (HRM) is alternative technique designed with characteristic strong, yet inexpensive and easy approach to analyze genomic regions with single nucleotide polymorphisms and variable loci which can also be used to determine the

spa locus diversity with high accuracy **(Mazi et al., 2015)**. This technique uses conventional PCR spa assay using oligonucleotides specific oligonucleotides modified and optimized for the for specific ThermoCycler instrument. All primers had an M13 sequence added to the 50-end of the gene specific priming sequence for subsequent sequencing. PCR and HRM have been performed in a single run **(Stephens et al., 2008)**.

2.9. Methods in identification of S. aureus

It is essential to distinguish *S. aureus* from coagulase-negative staphylococci (CoNS). Several methods are used to identify *S. aureus,* including production of protein A, cell-bound clumping factor, extracellular coagulase and heat-stable nuclease. In addition, molecular methods have been developed. Quality control uses both positive and negative control strains, or as directed in commercial systems in both identification systems. The screening of *S. aureus* is based on the growth on Mannitol salt agar (MSA) followed by a series of phenotypic methods. There is no single phenotypic test that can guarantee reliable results for the identification of *S. aureus*. Therefore, a series of tests have been devised for identification. *S. aureus* on Columbia agar with 5% defibrinated sheep blood produces colonies that are round, convex, and 1-4 mm in diameter with a sharp border. On blood agar plates, colonies of *S. aureus* are often surrounded by zones of beta haemolysis. The colonial appearance of golden colour is due to staphyloxanthin Swinement, a carotenoid Swinement and it is the etymological root of the bacteria's name, i.e. aureus = "golden" in Latin. This Swinement acts as a virulence factor, a bacterial antioxidant which helps to evade the reactive oxygen species of the host immune system. Mutant strains of *S. aureus* which lack staphyloxanthin least survive human neutrophils. MRSA strains, frequently appears with weak or no beta haemolysis and grows in medium with oxacillin and NaCl.

2.9.1. Phenotypic approach for identification of *S. aureus*

2.9.1.1.Coagulase tests

Tube coagulase test Free (extracellular) coagulase clots plasma in the absence of calcium. The tube coagulase test with rabbit plasma and examination of tubes after incubation for 4 hours and 24 hours is the standard test for routine identification of *S. aureus.* Tests negative at 4 hours should be re-examined at 24 h because some strains may require longer than 4 hours to form formation of clot. Some other species of staphylococci, including *S. schleiferi* and *S. intermedius,* may also give positive results. There are some rare strains of *S. aureus* that give negative results of coagulase tests. For routine testing more rapid tests are now widely used, particularly latex agglutination tests.

Slide coagulase test Clumping factor (bound coagulase) differs from free coagulase in that it is cell-bound and requires only fibrinogen. The slide agglutination test for clumping factor is very rapid but up to 15% of *S. aureus* strains are negative, therefore, all negative test results for slide tests should be confirmed with a tube agglutination test. Some species of staphylococci, like *S. schleiferi* and *S. lugdunensis*, may give positive results in here. This test is not suitable for bacterial isolates that are not easily emulsified and capsulated strained.

2.9.1.2. Latex agglutination tests

Commercial latex agglutination tests for *S. aureus* detected protein A and/or clumping factor. These tests had problems with some MRSA which produce little or no clumping factor and protein A. Later formulations of latex tests include protein A and/or clumping factor but also detect various surface antigens, which improved the sensitivity of the tests but at some expense to specificity due to cross-reaction with CoNS. In addition, any test including clumping factor may give false-positive results with *S. lugdunensis* and *S. schleiferi.* Staphaurex™ Latex Agglutination Test rapidly identify and differentiate staphylococci which possess coagulase and/or protein A, particularly *S. aureus.* Other commercial tests include; coagglutination tests—Sero-STAT®, Accu-Staph®, and Staphyloslide ®.

2.9.1.3. DNase and heat-stable nuclease tests

DNA hydrolysis test or Deoxyribonuclease (DNase) test, determines the ability of *S. aureus* to hydrolyze DNA and utilize it as a source of carbon and energy for growth. DNase agar, is a differential medium which is used for this test. The medium is pale green in color because of DNA-methyl green (indicator) complex. Methyl green is a cation that binds to the negatively-charged DNA. If the organism that grows in the medium produces Deoxyribonuclease, it breaks down DNA into smaller fragments. The breakdown of DNA results into to unbinding to the methyl green, and green color fades and the colony is surrounded by a colourless zone (**Kateete *et al.*, 2010**). Deoxyribonuclease (DNase) plates can be used to screen isolates but, several other species especially CoNS can be positives. Therefore, use of this test can be confirmed with an additional test. Heat-stable nuclease tests are used to identify *S. aureus*, although some rare coagulase-negative species can be positive. The metachromatic agar diffusion method for heat-stable nuclease devised to be used directly on blood cultures. A latex agglutination test based on heat-stable nuclease are available for use (**Mahon et al., 2015**).

2.9.1.4. Analytical profile index (APIstaph)

API strips give accurate identifications based on extensive databases and are standardized, easy-to-use test systems. The kits include strips that contain up to 20 miniature biochemical tests. All are quick, safe and easy to perform. API strips have a long shelf life, enabling every laboratory to keep the test kits on hand. Apiweb™ is a software product containing all of the API databases for a reliable automated interpretation of API strip results. It is rapid and easy to use by numerical profile of the strip acquired during identification of organism (**Koop *et al.*, 2012**). API Staph is a standardized system for the identification of the genera Staphylococcus, Micrococcus and Kocuria, which uses miniaturized biochemical tests and is adapted to a database. The complete list of those bacteria that are possible to be identified by this system can be found in the Identification table at the end of this package insert. The API

Staph strip consists of 20 microtubes holding dehydrated substrates. These microtubes are inoculated with a bacterial suspension, prepared in API Staph Medium, that reconstitutes the tests. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the Reading table and the identification is obtained by referring to the Analytical Profile Index or using the identification software, Apiweb™ **(Kloos et al., 1982)(Koop *et al.*, 2012)**.

2.9.1.5. Automated methods identification system

Automated systems including Vitek/Vitek2 (bioMe´rieux), Phoenix (Becton Dickinson) and Microscan (Dade Behring) include tests for methicillin/oxacillin susceptibility and are generally reported to be reliable for *S. aureus* although a small number of incorrect results, mostly false resistance, have been reported. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has recently been introduced in diagnostic microbiology laboratories for the identification of bacterial and yeast strains isolated from clinical samples (**Wolters *et al.*, 2011**). The fully automated VITEK 2 system can provide identification results for Gram-positive cocci in a few hours and the sensitivity has been improved by fluorescence-based technology, a major improvement currently compared to earlier versions of the same system (**Overdevest *et al.*, 2011**). However, these automated Identification systems are very expensive and may not be afforded by low resourced settings.

2.9.2. Molecular approach for identification of *S. aureus*

Several genotyping methods have been developed for discriminating different bacterial isolates and tracing infection origin. These methods utilize nucleic acids in the bacterial genome and characterizes species up to the strain level both phenotypically and genetically. This makes molecular typing the most important tool with high level of epidemiological traceability among isolates **(Sommerhalter, 2015)**. Efforts have been put in epidemiological investigations of MRSA due to its role as an important Hospital, community and livestock-acquired MRSA. The vigorous evolution and spread of MRSA has increased various strains

appearing in several regions rapidly. Therefore, these tools can help to better appreciate the evolution, dissemination patterns and AMR mechanisms. This is prerequisite for infection prevention and control response. Notable, PCR-based methods includes pulsed-field gel electrophoresis (PFGE), coagulase gene PCR-restriction fragment length polymorphism (RFLP) and Staphylococcal cassette chromosome mec typing (SCCmec) has played an important role in molecular typing **(Lin *et al.*, 2010)**. However, sequence-based techniques like spa gene-typing and multilocus sequence typing (MLST) has vital role in genotyping **(Chao et al.,2014)**. PFGE and MLST that are considered as 'gold standard' in typing of MRSA, despite the cost and laborious nature of these techniques.

2.9.2.1. Amplified fragment length polymorphism (AFLP)

This is a PCR-based method used for DNA fingerprinting and uses restriction enzymes (e.g. endonuclease) to cut the genomic DNA and as a result, the double-stranded oligonucleotide adaptors that comprises of a core sequence and an enzyme-specific sequence, are bound to the sticky ends of the restricted fragments. The PCR ThermoCycler is employed to amplify the restricted fragments ending with the adapter selectively, using primers complementary to the adapter sequence. The restriction site sequence and additional nucleotides from the end of the unidentified DNA are designed. The restriction fragments usually, 50–100 bps are amplified using florescent dye-labelled PCR primers and detected by size using automated DNA sequencer. Gel electrophoresis can also be used to visualize and analyze the amplified fragments. Upon analysis, a high-resolution band is produced via computer to reflect the genetic relatedness among bacterial isolates **(Dashti *et al.*, 2009)**. AFLP technique has a higher discriminatory power in comparison to PFGE, and it is known to provide greater genetic resolution. This method can also group MRSA isolates into clusters according to the clonal complexes (CCs) with high reproducibility than PFGE and MLST. However, it does not discriminate different sequence types (STs) or spa types within CC, laborious and expensive **(Mazen M., 2018)**.

2.9.2.2.16s ribosomal RNA (16s rRNA) typing

This method uses 16S rRNA which comprises ~1500pb nucleotide sequence coding for catalytic RNA that is part of the 30S ribosomal subunit. 16S rRNA gene is consist of nine variable regions (V1–V9/30– 100 bp long) (**Salipante *et al.*, 2013**). It has a sequence diversity among different bacterial species, thus, permitting identification purposes. V1–2-3 regions are positioned at the 50 end of the 16s rRNA gene which known to be appropriate and more sensitive than other regions for inferring different types of bacteria. This gene is constant and stable in function, making it an important candidate for use in determination of phylogenetic relationships. It is less time-consuming and labour intensive compared to culture-based approaches. The short read lengths, variances ascending from the diverse regions selected, sequencing errors and difficulties in evaluating operational taxonomic units on addition to being a single marker makes 16S rRNA usage a challenge to assess the bacterial diversity. However,16S rRNA combined with mecA using multiplex PCR, is considered as useful tool for rapid characterization of MRSA **(Mazen M et al., 2018)**.

2.9.2.3.Staphylococcal cassette chromosome mec typing (SCCmec)

SCCmec comprises of essential elements which can be detected by regular PCR. This includes, ccr genes (ccrA and ccrB) and mec gene complex (mecI and mecRI). There are 11 major types of SCCmec elements (I–XI) which have been identified **(Berglund *et al.*, 2005).** There are four allotypes of ccr complex (types 1, 2, 3 and 5) and three classes of mec complex (A, B and C). Different groupings of these complex classes and allotypes generate various SCCmec types. SCCmec elements are currently classified into types I–V basing on the nature of the mec gene complex and ccr allotypes. This characterization method is used to distinguish HA-MRSA and CA-MRSA typing, where SCCmec types I, II, III and VIII are usually acquired by HA-MRSA, while SCCmec types IV, V, VI and VII are acquired in CA-MRSA. Thus, it is very useful and important molecular tool in understanding the potential epidemiological background of the strains **(Mazen M et al., 2018)**.

2.9.2.4. Multiple-locus variable-number tandem repeat assay (MLVA)

The method makes use of variable-number tandem repeat (VNTR) polymorphism and involves PCR amplification of five specific loci (sdr, clfA, clfB, ssp and spa) composed of seven individual genes (sdrCDE, clfA, clfB, sspA, spa, mecA and fnbP). Amplification is through multiplex PCR, followed by agarose gel electrophoresis to separate the amplified bands and comparison of the band patterns. This helps to identify strains and genetic clusters or clones among MRSA (Verkade *et al.*, 2014). This method can determine the genetic diversity and evolutionary lineage with discriminatory power and reproducibility. However, in a highly conserved genomes, there may be sufficient DNA polymorphisms in these limited sequence targets to exhibit alleles. Also, small deletions and insertion in the regions flanking the repeat units may result into misinterpretations, making the MLVA results ambiguous **(Mazen M et al., 2018)**.

2.9.2.5.Restriction fragment length polymorphism (RFLP)

The restricted enzymes are used in this method to detect the variations in homologous DNA fragments. The DNA fragments are amplified using regular PCR, subsequently these fragments are separated by gel electrophoresis based on length of the fragments, importantly, coagulase gene (coa) and Staphylococcal Protein A (spa) gene. PCRRFLP typing of coa gene discriminate *S. aureus* strains basing on sequence variation within the 3′ end coding region of the gene. The discriminatory power of PCRRFLP tying of coa gene depends on the heterogeneity of the region consisting of 81 bp tandem repeats at the 3′ coding section of the coa gene **(Bosch *et al.*, 2013)(Hasman, 2009)(Mazen M *et al.*, 2018)**.

In addition, the region X comprises of up to 12 elements each with a length of 24 nucleotides and it is highly polymorphic. Variety of X region causes protein A variation can be detected by the number of repeats in the region X of spa. As a result, the PCR-RFLP assays (coa and spa RFLPs) are useful molecular markers for a rapid, and initial study of MRSA outbreaks. It has good discriminatory power, typeability and reproducibility in MRSA typing and

therefore, used to in routine infection control proGram in health care systems as well as epidemiological investigations **(Mazen M et al., 2018)**.

2.9.2.6.Staphylococcal protein A (spa) typing

Typing Staphylococcal protein, A (spa) is a sequence-based method that targets VNTR of the spa gene region encoding protein A. The spa gene region is polymorphic because of spontaneous mutations due to loss or gain of sequence repeats. Spa gene typing is reported to be a highly effective tool in subtyping both MSSA and MRSA. Sequenced data can be evaluated by means of free accessible offline bioinformatics tool known as Ridom Bioinformatics (Ridom, GmBH, Germany) (http://spaserver.ridom.de) **(David *et al.*, 2013)**. This spa server database provides global frequencies and information related to mapping of spa with the MLST *S. aureus* database. There are 748 diverse repeats with more than 17,416 spa types which have been described from 131 countries with a total of 384,806 strains (http://spaserver.ridom.de). High quality sequences are synchronized with spa server (Ridom server) specifically for spa typing, providing a typical worldwide nomenclature together with integral quality control. Subsequently, Based Upon Repeat Pattern (BURP) algorithm can be used to analyze the diverse spa types associated to each other which provides a good consistency with MLST-CCs, where ST that shares at least five of seven identical alleles are grouped into a single CC. This is advantageous due to ease of interpretation of data generated, less time consuming, less laborious, highly reproducible and highly comparable. It is also able to detect both slowly and rapidly accumulated molecular variations as well as outbreaks investigation and molecular evolutions of population structure. HA-MRSA, CA-MRSA and livestock MRSA (LA-MRSA) spread can be monitored with spa typing in epidemiological studies **(Mazen M et al., 2018)**.

2.9.2.7.Multilocus sequences typing (MLST)

Multilocus sequence typing (MLST) method characterizing bacterial isolates on the basis of the sequences of ~450-bp internal fragments of seven housekeeping genes. For each gene

fragment, the different sequences are assigned as distinct alleles, and each isolate is defined by the alleles at each of the seven housekeeping loci (the allelic profile or sequence type [ST]). As there are many alleles at each of seven loci, isolates are highly unlikely to have identical allelic profiles by chance, and isolates with the same allelic profile can be assigned as members of the same clone. Sequence data are readily compared between laboratories, and a major advantage of MLST is the ability to compare the results obtained in different studies via the Internet **(Hudson *et al.*, 2013)**. In addition, the data obtained by MLST can be used to address basic enquiries on the phylogenetics of bacterial species. MLST is still considered as the rapid method for subtyping for MRSA producing results that are comparable between laboratories using MLST server for interpretation **(Mazen M et al., 2018)**.

2.9.2.8.Pulsed-field gel electrophoresis (PFGE)

This approach is used to detect the dispersion of large segments of DNA using gel with high electrical fields that facilitate changing in DNA direction periodically. The molecular sieve of gel is used to transform DNA from cathode to anode using common electrophoresis method. Two electric fields are used in PFGE technique, which allows changes in the direction of the DNA **(Lin *et al.*, 2010)**. Ethidium bromide dye is used to differentiate the DNA band spectrum. In terms of *S. aureus*, isolation of intact bacterial chromosomes is required prior to PFGE procedures, where the chromosomes are broken down into large DNA fragments using cutting restriction endonuclease such as SmaI. Later, DNA fragments can be separated via agarose gel "pulse-field" electrophoresis, where those separated DNA fragment could be monitored as a banding pattern in the gel. The gels are dyed and captured by an imaging system and analysed using BioNumerics software proGrams with the Dice coefficient and Un-weight pair group matching analysis (UPGMA) and graphical dendroGram is generated by DNA fingerprinting software. PFGE has a higher discriminatory power than PCR-RFLP of coa gene and other PCR-based fingerprinting methods as it enables the entire chromosome to be analysed rather than selected (random) portions of chromosome.

PFGE is considered as the "gold standard" genotyping method. It has high reproducibility due to due to the standardized of protocols. and standard interpretation guidelines. However, the method is time time-consuming, labour-intensive and has insufficient resolution power to differentiate bands of identical size, requiring highly skilled operators **(Mazen M et al., 2018)**.

2.9.2.9.DNA microarray typing

The method uses a collection of DNA probes that are attached to a solid surface in ordered manner. Ideally, complementary nucleotide sequences for specific bacterial isolates are detected by DNA probes. This approach is specific tools to identify several genes for specific bacterial strains. It can also be used to identify allelic variations of a gene which exists in all strains for particular species. Usually, target DNA could be labelled by chemical, enzymatic reaction and DNA microarray hybridization **(Brennan *et al.*, 2012)**. Then, labelled target DNA and an immobilized probe create signal due to successful hybridization, giving measurement automatically using scanner. This typing method is extensively used to analyze genomic mutations such as single-nucleotide polymorphisms (SNPs). DNA microarray technology is highly accurate, but the reproducibility data needs to be established to the broad application of this technology, and it is not practical if the target of typing is SNPs of highly clonal species **(Mazen M et al., 2018)**.

2.9.2.10. Whole genome sequencing (WGS)

This technique is named second generation to differentiate it from first generation sequencing based on the Sanger method. It has ability to create millions of reads (35–700 bp length), and advantage over several traditional sequencing methods. The nucleotide sequence of the genome is assembled by gathering numerous short sequence reads from overlapping regions, or comparison with previous reference sequences genomes (re-sequencing). WGS is an exceptional tool for bacterial identification and characterization. However, interpretation and computation of the huge data set generated is the main chailenge **(Lindsay, 2014a)**. The

method can be used to determine the genetic relatedness between bacterial isolates based on sequence analysis of the whole genome. In addition, WGS has the ability to discriminate numerous genomes within an SNP, which cannot be attained by conventional molecular typing approaches. Various phenotypic characteristics such as virulence and AMR genes can also be inferred by WGS technique. WGS could permit determination of emerging infectious strains and genetic diversity among the *S. aureus* **(Mazen M et al., 2018).**

2.9.2.11. Polymerase chain reaction (PCR) point of care technologies

2.9.2.11.1. The GeneXpert MRSA/SA BC Assay

The GeneXpert MRSA/SA BC Assay (Cepheid, Sunnyvale, CA) is a real-time PCR - based method that can recognise *S aureus* and MRSA from blood cultures or other body fluids within about 1 hour of a positive signal from automated blood culture system **(Oh *et al.*, 2013)**. This technology depends on the detection of the staphylococcal protein A gene (*spa*) for identification of *S. aureus* and deduces methicillin resistance through simultaneous detection of the methicillin-resistance gene *mecA* and the junction between the SCC that harbours *mecA* (SCC*mec*) and the *S. aureus* genome (*orfX*). All the three targets must amplify in order to deem the specimen positive for MRSA. The method is sensitive due to side stepping of pitfalls of some other PCR-based assays that target only the SCC*mec* junction, which can falsely detect MSSA strains with cassette chromosomes lacking *mecA* (empty cassettes or "*mecA* dropouts") as MRSA **(Spencer et al., 2011)**. The assay cannot differentiate bacterial strains and therefore not suitable for determining MRSA phylogenetics but rather clinical usage.

2.9.2.11.2. The FilmArray platform

The FilmArray platform (BioFire, Salt Lake City, UT) is a closed diagnostic system that allows high-order multiplex PCR analysis synchronised with automated readout of results directly from positive blood or other body fluid cultures in 1 hour. This diagnostic system combines nucleic acid extraction from clinical specimens, high-order nested multiplex PCR,

and post-PCR DNA melting curve analysis. Extraction, amplification, detection, and analyses are all completely automated within the commercially provided pouch. Results of the assay are provided by the software only if the quality control reactions are passed. Each pouch includes two internal run controls for both the primary amplification and the analyte-specific detection stages. When these two internal control fails, the result is listed as "invalid." The FilmArray blood culture identification (BCID) panel, is capable of identifying 19 bacteria, five yeasts, and three antibiotic resistance genes including mec A gene for MRSA and VanA and van B genes for vancomycin resistance. FilmArray assay is a rapid identification method, reproducible and demonstrates high sensitivity and specificity **(Altun *et al.*, 2013)**. The assay is suitable for rapid detection of blood borne or other body fluid selected bacterial and fungal pathogens but cannot be used to determine the relationship among strains.

2.10. Management of MRSA

2.10.1 Management of CA – MRSA

CA-MRSA strains, like all MRSA, are resistant to all beta-lactam antibiotics, including flucloxacillin, amoxycillin/clavulanate and all cephalosporins **(Weese, 2010)**.. Incision and drainage (I&D) is recommended, whenever possible, as a priority for management of skin and soft tissue infections (SSTIs). I&D alone may be adequate for minor abscesses and boils in absence of systemic symptoms or co-morbidities. Antibiotics can be prescribed if I&D is impossible, or there if significant co-morbidities on a patient. Patients with large or deep abscesses, may require hospital management especially those with extensive cellulitis, or who are unwell with systemic symptoms (**Coates *et al.*,2009**).

Decolonization may be obtainable when individuals or respective household contacts have recurring CA-MRSA or staphylococcal infections or at risk for acquiring staphylococcal infection particularly those with chronic skin disorders, diabetes, peripheral vascular disease or immunosuppression (**HPSC, 2013**). In addition, healthcare workers or carers, school

children and those in day care Centre or sports club are also at risk of acquiring CA – MRSA. Decolonization because strains of CA-MRSA are of particular concern due to their increased transmissibility and antibiotic resistance (**Coates *et al.*,2009**) . Household infection requires decolonization of all entire family members in the house, even if some members may not have active infection **(Cirkovic *et al.*, 2015)**.

MRSA are resistant to all the beta-lactam antibiotics (e.g. flucloxacillin, amoxycillin/clavulanate) and all cephalosporins (e.g. cephalexin). Antibiotic recommendations related specifically to skin and soft tissue infections, and not to other infection types may include Clindamycin (1st line) TMZ-SXT (2nd line) and Doxycycline (3rd line). However, the decision to prescribe these antibiotics requires clinical judgment and an assessment of the severity of the infection **(Loomba *et al.*, 2010)**. Longer therapy may be required for carbuncles and those with associated cellulitis. Where TMZ –SXT is not appropriate particularly MRSA is resistant or allergies, clindamycin capsules (2nd line antibiotic) cannot be considered, the use erythromycin ethyl succinate oral suspensions considered as long as MRSA is sensitivity to erythromycin. Note that in neonates (<2 months) Erythromycin is specifically contraindicated due to increased risk of pyloric stenosis **(Mendes *et al.*, 2017)**.

2.10.2. HA - MRSA treatment

As of today, there are an increasing number of agents available for the treatment of MRSA. However, increasing antibiotic resistance is a public health challenge **(Weese, 2010)**. Vancomycin, an acceptable treatment option for MRSA is facing a serious resistance by some strains, this has created an urgent need for new antimicrobial **(Whelan *et al.*, 2011)(Stefani *et al.*, 2012)**. Daptomycin sought to treat MRSA bacteremia has been associated with high clinical failure rates requiring a combination therapy to improve outcomes **(Reygaert, 2013)(Mendes *et al.*, 2017)**. Some strains with decreased susceptibility to vancomycin may

also show decreased susceptibility to Daptomycin **(Loomba *et al.*, 2010)(Micek, 2007)**. Lipoglycopeptides have been suggested to be excellent antibiotic with in vitro potency against MRSA. However, the cost, long half-lives and complex pharmacokinetics has limited its routine use in the under resourced settings **(Mendes *et al.*, 2017).** Linezolid is the most important option against MRSA infections **(Cirkovic *et al.*, 2015)(Rasmussen *et al.*, 2012)**. The cost of Linezolid in local settings account for its limitations in routine clinical use **(Filice *et al.*, 2010)**.

2.10.2. MRSA prevention and control

MRSA is highly infectious, and carriers have been observed to spread the organism to the environment at a higher rate compared to other bacterial infections, while contaminating the environment. Kavanagh et al observed that MRSA colonized patients often contaminate their environ within 33 hours leading to such surfaces contaminated for up to one to two months as the source of MRSA **(Kavanagh et al., 2018)**. In addition, MRSA can be endemic in the general population as carriers including those that visits health care settings at the outpatient units. Also, healthcare workers can transmit *S. aureus* to patients and poses a risk of spreading pathogens to the environment **(Russotto et al., 2015)**.

MRSA rapid spread calls for the reassessment of personal and institutional hygiene. Since MRSA presents with an urgent medical problem due to its association with difficult to treat antimicrobial resistance infections, there is an argent need to take infection control precautions **(Rasmussen *et al.*, 2012)**. Hygiene has social and procedural rules that prevent MRSA transmission which results into significant reduction in the spread **(Jarvis, 2010)**. Any prevention and control approach must be strengthened by changes in attitude, embraced by all personnel in hospital facility setting **(SARI, 2005)**. The major components of preventing and controlling MRSA forms integral part of standard precautions which include hand and environmental hygiene, patient isolation strategies, and patient/staff decolonization **(Jarvis, 2010).**

Screening of MRSA to prevent surgical site infections in preoperative patients is an important practice that can result in early success in prevention of MRSA regarding its continued progression and spread of antibiotic resistance within the hospital settings, with additional resistance developing to other antibiotics including vancomycin **(Kavanagh *et al.*, 2018).** Routine screening to patients who are ready for discharge from the hospital or transfer to another healthcare facility, care should be put to consideration and emphasized. Individuals carrying MRSA should be prescribed with antiseptic washes, Nasopharyngeal ointment and mouthwashes to increase chances of removing the MRSA (**HPSC, 2013**). Policy to screen and restrict healthcare employers from assigning healthcare workers in patient contact areas while they are carriers of dangerous pathogens. This policy should apply to MRSA carriers not only because of the pathogen's resistance to antibiotics but also because of concerns regarding increase virulence. MRSA-positive staffs if identified should be restricted from their professional activity depending on the nature of their work. Finally, politicians and others need to commit to providing the necessary resources to maximize MRSA prevention and control. Protocols for screening and decolonization should be instituted and adopted, implemented in every healthcare setting. The protocol commonly used for *S. aureus* decolonization is daily body washes with an antiseptic for five days in addition to an intra nasopharyngeal antibiotic ointment, given three times a day (**Coates *et al.*,2009**). Individuals with MRSA positive patients, 2% mupirocin can be applied intra nasopharyngeal while those with MSSA positive patients should be administered with naseptin Nasopharyngeal Cream (chlorhexidine hydrochloride 0.1%; neomycin sulfate 0.5%)(**HPSC, 2013**). However, universal decolonization of all patients or healthcare personnel regardless of their carrier state should be avoided because of the potential to spread resistance **(Kavanagh *et al.*,2018)**.

S. aureus is the leading cause of purulent bacterial infection in humans and livestock and has potential for local dissemination causing lesions in several tissues and anatomical sites. It can spread the infections and either acquired in the community or in association with health care

/estock animals. The situation of *S. aureus* as one of the significant human and livestock
erial pathogen, essentially due to its virulence potential and ubiquitous nature to colonise
ans and livestock and should never be ignore in the microbial realm.

CHAPTER THREE: METHODOLOGY

3.1. Study site

Geographically, the study was conducted in greater Kabale region, located in the southern part of the Western Region in the republic of Uganda. Greater Kabale has three districts of; Rubanda, Rukiga, and Kabale, with over 19 sub-counties **(Nyangoma, 2014)**. In this region, grazing land is gradually shrinking due to population pressure (**Turinawe *et al.*, 2014**), a phenomenon that has resulted into increased close contact between man and domestic animals. In such interaction, both subjects are predisposed to infections from one or the other as the consequence. The distribution of livestock within the district is almost even except for Kamwezi sub-county in Rukiga County which has a slightly higher concentration. The district has about 70,000 heads of cattle, about 40,000 goats, 30,000 sheep, 10,000 Swines and 100,000 poultry. Out of the 70,000 heads of cattle about 2,500 (about 3.6%) are exotic (mostly Friesian) and 7,500(about 11%) are grades, the rest being indigenous. The land area devoted to grazing is estimated to be 12% of the total arable land in the district ***(Turinawe et al.*, 2014).**

Kabale regional referral hospital (KRRH) is a 300 bed capacity hospital and is located in the Kabale municipality. The daily OPD attendance is approximately 250 – 300 patients and the inpatients are averagely 400 patients (**DHIS, 2014**) with average bed occupancy of 98% (**SUSTAIN, 2013**). The annual inpatient admission is approximately 18000 and annual outpatient department attendance is averagely 15600 (**SUSTAIN, 2013**). The hospital serves as a teaching hospital for Kabale school of comprehensive nursing (KSCN) and Kabale institute of health sciences (KIHS). It is among the referral hospitals where medical students go for internship and industrial training and therefore has a large number of students.

3.2. Study population

The population used in this study involved human and animal. Among the human; included outpatients of all ages and sex that meet inclusion criteria and their respective household

members whose outpatient were MRSA positive. Inpatients of all ages, sex and hospitalized for five days or more whose clinical specimens has been sent to the laboratory for microbiological investigations. For animal population included all household swine and cattle of all ages, belonging to the family where the outpatients with confirmed presence of MRSA in their nasopharynx came from.

3.3. Study design

This was a cross sectional study conducted in Kabale district (south western Uganda). The study consisted of two parts, human and animal study. Human study included; outpatients, admitted patients (in patients) and house hold contacts, whereas Animal subjects included cattle and swine from respective homesteads.

3.4. Sampling techniques

Humans and animals were used as subjects for this study from where specimens for analysis were drawn. Several sampling techniques were employed in this study. Among the outpatients, a convenient random sampling technique was used where a research administered questionnaire was subjected those who consented and met the inclusion criterion. The household contact sampling was determined by the number of outpatients with MRSA. Only those outpatients found with MRSA, were followed back to their homes and all the household contact were requested to consent prior to be given research administered questionnaire followed by sample collection. At every homestead, a simple random sampling was applied while targeting those who are frequently in contact with domestic animals.

A simple random sampling technique was subjected to animals, and only those homes whose members that visited the hospital outpatient department with any ailment complaint and tested positive for MRSA. Consent and permission to collect specimen from domestic animals (cattle or swine) was sought from the owners. A home with both cattle and Swines or any of the two were allowed to participate. Animal swabs specimens preferred were collected

from the udder tits and Nasopharyngeal for cattle and the snout for the swine as these areas are likely to get in contact with the human owners. Any wound observed during the sample collection, was also considered for swabbing. Convenient random sampling was also used to collect specimen from the domestic animals with the help of the farmers.

Among the inpatients, only the clinical specimen that reached the laboratory for microbiological testing and met inclusion criteria were all randomly selected and tested.

3.5. Study specimens

The study specimens that were considered for the study are presented below;

Subjects		Specimens
HUMANS	Outpatients	Nasopharyngeal swabs
		Wound swabs (if any)
	Homestead members (community)	Nasopharyngeal swabs
	Inpatients	Pus, urine, sputum and blood
	Health care workers	Nasopharyngeal swabs
ANIMALS	Cattle	Nasopharyngeal and udder swabs
	Swine	Nasopharyngeal and skin swabs

3.6. Sample size

Minimum sample size required to accurately assess outpatient MRSA carriage was calculated as 384 on the basis of expected prevalence of Nasopharyngeal colonization according to **David *et al.*, 2010,**where it was estimated to be at 51%. In addition, **Kateete *et al.*, 2011** estimated the prevalence of HA – MRSA to be 46%, and therefore, this translates into in patient sample size of 382 through calculation using **Kish Lisle (1965** formula; $n = z2\ p\ (1-p) / d2$. Where, z = Score for 95% confidence interval = 1.96; p = Prevalence (To estimate the proportion of MRSA infections); d = Sampling error that could be tolerate= 5%.

1-p= Probability. The sample size of 384 out patients attending KRRH were further translate into 1536 household contacts (from 4 x 384) since the average number of people per homestead is approximately 04 according to **NPHC, 2011).** In addition, according to **MAAIF, 2008** census, each household has an average of 04 cattle, and 01 Swine. Therefore, we estimated 1232 cattle and 308 Swines which was included in the study within Kabale district. Such large sample sizes were chosen in order to maximize the chance of uncovering a specific mean difference, which is also statistically significant and therefore reliable.

3.7.Inclusion and exclusion criteria

3.7.1. Inclusion criteria

This study included all those people of all ages and sex reporting to KRRH outpatient department (OPD) with a health complaint and residing in Kabale district and had more than one member of the household (HH) as well as domestic animals particularly swine and cattle or both at their respective homes. Inpatients of all ages and sex admitted at KRRH for five days or more and whose clinical samples were collected and sent to the laboratory for microbiological investigations as part of routine clinical work at their respective wards were also included in the study. Only clinical samples accompanied with patients laboratory requisition forms bearing patients names, age, sex, admission date, antibiotics being used and type and source of specimen was included in the study.

3.7.2. Exclusion criteria

All those people reporting to KRRH with a health complaint but not residing in Kabale district and those residing in the mentioned district but without any domestic animal at home were excluded from the study. All HH, whose outpatients turned out to be negative for MRSA were excluded from the study. Domestic animals (cattle and swine) that belonged to those outpatients whose nasopharyngeal swabs turned out to be negative for MRSA were not Included in the study. In addition, domestic animals which are not swine or cattle were not included in this study. Patients admitted in the same hospital for less than 5 days were also

excluded in the study. Clinical specimen that reached the laboratory with partially filled laboratory request forms were rejected and not included in the study.

3.8. Study flow

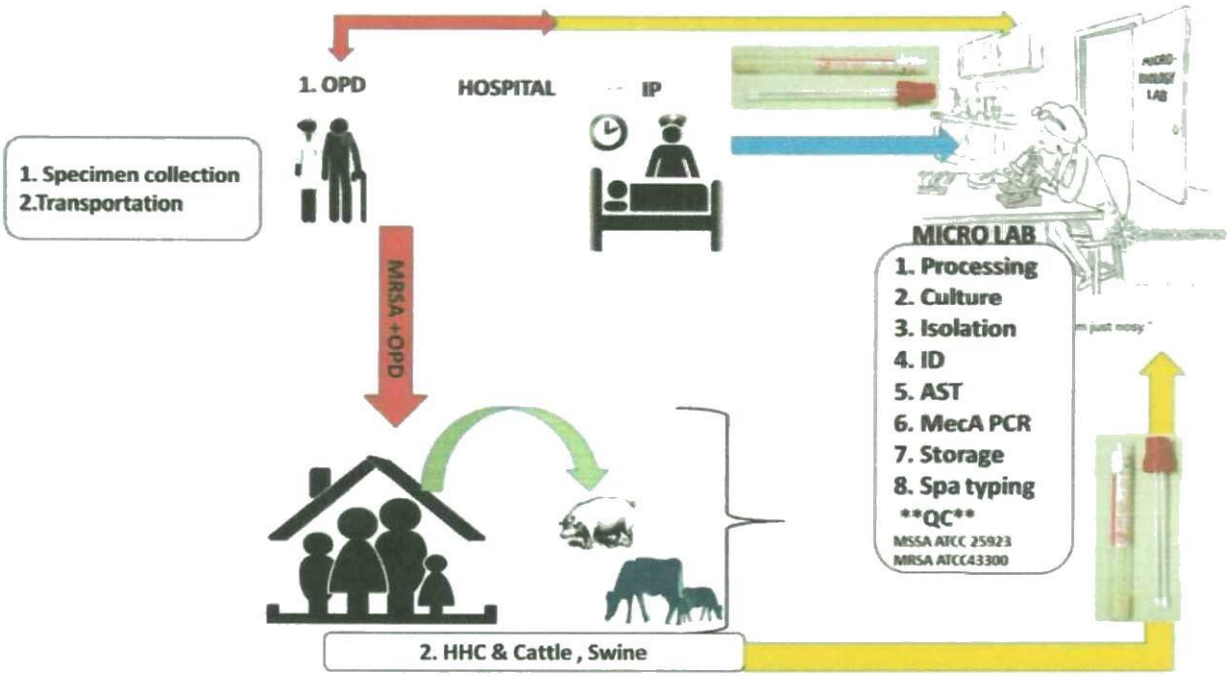


Figure 7. Flow of the study.

The study started at outpatient department of KRRH, where nasopharyngeal swabs of those outpatients with household domestic animals were corrected and tested for presence of MRSA. Positive MRSA patients were followed back into their homestead where the house hold members including domestic animals were swabbed and tested for MRSA. In patents were independently tested for presence of MRSA using routing clinical samples.

3.9. Laboratory methods

3.9.1. Specimen collection and transportation and culture

After consent, samples from anterior nares of the patients from the OPD were collected using two sterile cotton wool swabs moistened with nutrient broth. Age, sex, family size and the number of cattle, swine or both in their homes were noted. The Nasopharyngeal swabs were transported in Amie's transport medium - ATM (Hi-Media, Mumbai) and processed within 6

hour where, these swabs were immediately inserted into Nutrient broth - NB (Hi-Media, Mumbai) with 7.5 per cent NaCl (Difco, Detroit, MI) with slight modification of **Chatterjee *et al.* 2009** methodology to suit our laboratory environment. The NB was incubated overnight for enrichment of *S. aureus* and subcultured onto mannitol salt agar (Difco, Detroit, MI). For inpatients, their specimen included wound swabs, urine and sputum and aspirates that were sent to the laboratory for routine microbiological investigations. These specimens were collected from hospitalized patients during routine clinical work and transported to the laboratory within hours for further processing and culture to isolate *S. aureus*. Domestic animals or Cattle Nasopharyngeal swabs, lactating cow's udder teats swabs were collected and placed into ATM (BD, USA) for transportation to the laboratory. Wound swabs (if any) were collected and transported to the laboratory for analysis.

3.9.2. Identification and isolation of *S. aureus*

S. aureus isolation and its phenotypic identifications were carried out in the Microbiology department at Central Public health laboratories (CPHL) – Ministry of Health Uganda. The specimens were inoculated on to Mannitol salt agar (Difco, Detroit, MI) and incubated at 37°C using aerobic incubator (Memmert GmbH) for 24 - 72 hours. *S. aureus* was identified using a range of identification tests which included colony morphology on MSA, Catalase, coagulase and DNase test as well as Staphaurex™ plus latex agglutination test. In details, a single morphologically distinctive of Mannitol fermentation positive colony was emulsified in sterile saline in comparison with 0.5 McFarland standard and further subcultured using 0.2µL size sterile disposable plastic loop (ThermoFisher scientific) on Blood agar plate (BAP) as purity plate which was further incubated aerobically for 18 – 24 hours. The colonies on BAP were inspected, isolated and identified for catalase production using 3% hydrogen peroxide (Human diagnostics) and Staphaurex™ Plus Latex Agglutination Test (Thermo Scientific™) following manufacturer's instructions.

3.9.3. Antimicrobial susceptibility testing

To infer and inform clinical and veterinary management setting in the prescriptions of antibiotics, all confirmed *S. aureus* isolates were tested for antibiotic susceptibility using disc diffusion technique modified to suite our environment. The selection of antibiotics tested was based on the routine usage in both health and veterinary settings in the region. These antibiotics included; amoxicillin (10 µg), erythromycin (15µg), clindamycin (2µg), rifampicin (5µg), ceftriaxone (30µg), gentamycin (10µg), trimethoprim-sulfamethoxazole -TMP – SMX (1.25/23.75µg), tetracycline (10 µg), ciprofloxacin (5µg) and vancomycin (30µg). We emphasized on placing of erythromycin (15µg) disk next to clindamycin (2µg) in a 15 – 26mm distance apart to quickly detect Inducible resistance to clindamycin by the 'D – test', where flattening of the zone of inhibition adjacent to erythromycin disk was regarded as D - test positive. The zone of inhibition was interpreted following **CLSI, 2017** guidelines. The similar described procedure was always applied on to *S. aureus* - MSSA (ATCC 25923) and *S. aureus* – MRSA (ATCC 43300) as negative and positive controls respectively on to every new prepared batches of MSA, MHA, Brain heart infusion broth supplemented with NaCl (7.5%w/v) and BAP as part of quality control (QC). Media QC forms were filled and filed regularly to monitor quality of the media and reagents used. Study results were recorded in the register and data extraction form which was further transferred to excel spreadsheet for further statistical analysis.

3.7.4 D- test performance for screening of inducible clindamycin resistance isolates

Each S. aureus isolate that was found to be resistant to erythromycin was tested for inducible resistance by 'D test' according to CLSI guidelines **(CLSI), 2017).** Suspension of the isolated organism equivalent to 0.5 McFarland standard turbidity was seeded onto MHA plate (Difco Laboratories, Detroit, USA). Clindamycin (2ug) and Erythromycin (15ug) antimicrobial disks (Oxoid™) were positioned at a distance of 15mm (edge to edge) from each other. Quality control was performed by S. aureus ATCC 25923. Following overnight incubation at 37°C, a

D-shape zone around the clindamycin in the area between the two disks an indication for inducible resistance.

3.10. Phenotypic and Genotypic detection of MRSA

The *S.aureus* isolates together with control strains (MSSA ATCC 25923 and ATCC 43300) were further tested for phenotypic methicillin resistance using oxacillin (6µg) and Cefoxitin 30ug (Oxoid™) disk diffusion method according to CLSI guidelines (**CLSI, 2017**). The phenomenon of heteroresistance within *S.aureus* was eliminated by incubating all inoculated MHA plates at 35° C for a full 24 hours before reading (**Skov *et al.*, 2014**). All isolates were stored in 6% glycerol for feature retrieved during Molecular analysis. The DNA of the phenotypically detected MRSA isolates and control strains (MSSA ATCC 25923 and ATCC 43300) were extracted according to **Kumurya(2015)**, which was further genotypically tested for presence of MecA gene using polymerase chain reaction (PCR) followed by Gel electrophoresis.

3.9 Packaging and storage of MRSA and MSSA isolates

All the MRSA were stored in solution of 30% glycerol (v/v). Storage was made by suspending 3 -5 MRSA colonies from BAP into 2mL 30% glycerol (v/v) in cryogenic tube (Merck®). The mixture was vortexed using laboratory Vortex mixer (Thermo™ Scientific) to get homogenous solution. The tubes were then labeled well and stored at - 80°C temperature in a freezer (Thermo™ Scientific).

3.10 Spa typing and High-resolution melting analysis for MRSA

692 MRSA isolates were collected from inpatients (n = 223), outpatients (n = 104), Household community contacts (n = 160), swine (n =22) and Cattles (n = 170). HRM-based real-time PCR spa assay was performed on single MRSA colony using SensiMix™ HRM kit and melting temperatures (Tm) were analyzed by the Rotor-Gene 6000 proprietary software. Selected strains were also subject to DNA sequencing for confirmation and sequences were analyzed using BioNumerics software.

All MRSA isolates were confirmed using Mec A polymerase chain reaction(PCR) and their respective DNA extraction using QIAampDNA MiniKit (Qiagen GmbH, Hilden, Germany) for genomic DNA extraction according to the manufacturer's instructions. Lysostaphin (Sigma Aldrich, US) was used to a final concentration of 15 µg/mL for cell wall lysis. The concentration and quality of the purified gDNA was determined by UV spectrophotometry at 260 and 280 nm. Spa typing was performed as described by **Mayerhofer et al., 2015.** Spa gene PCR products were subjected to DNA sequencing for both strands by Macrogen (Seoul, South Korea) where the conventional PCR *spa* assay using oligonucleotides 1113F (5'-TAAAGACGATCCTTCGGTGAGC) and 1514R (5'- CAGCAGTAGTGCCGTTTGCTT) (www.ridom.de/doc/Ridom_spa_sequencing.pdf) was adapted and adjusted for the LightCycler LC480 appliance (Roche Diagnostics, Penzberg, Germany). All primers had an M13 sequence added to the 5'- end of the gene specific priming sequence for subsequent sequencing. PCR and HRM were performed in a single run. In a final volume of 10µl the HRM PCR reaction mix contained 5 ng of sample gDNA, 0.35 pmol of each primer, 3 mM $MgCl_2$ and the Light Cycler 480 High Resolution Melting Master using ResoLight high-resolution melting dye (Roche Diagnostics, Vienna, Austria). PCR conditions were as follows: 10-minute activation at 95°C, 45 cycles 95°C for 10 seconds, 60°C for 10 seconds and 72°C for 10 seconds and a final step at 95°C for 1 minute and cooling to 40°C for 1 minute. HRM was performed from 60°C to 95°C, rising at 0.02°C/s with 25 acquisitions per degree. All reactions were performed in quadruplicate using an epMotion work station (Eppendorf, Hamburg, Germany) for automatic sample preparation in 384-well microtiter plates (LC plate, Roche Diagnostics, Vienna, Austria).

Additional DNA was subjected to the reaction mixture (spiking) to separate spa types demonstrating similar or even indistinguishable melting curve profiles to increase the overall quality of the assay. Spiking resulted into the development of heteroduplex amplicons with melting curve profiles evidently unique from those of homozygous amplicons. Finest

outcomes were attained by adding 0.5 ng gDNA of *spa* type t003 and 0.5 ng gDNA of *spa* type t030 to the reaction mixtures. We used the LC 480 gene scanning software version 1.5 with manual settings for sensitivity at 0.30, for temperature shift at threshold one for HRM curve analysis. After control and normalization as well as temperature shifting the melting curves, difference plots were produced by choosing one HRM curve profile as the starting position(baseline). Only amplification products that reached the plateau phase were collected and analyzed. For optimal performance (i.e. correct assignment of melting curve profiles to a known sequence) of HRM experiments, each run had to contain well-characterized standards with known sequences. This assisted us to reliably compare melting curves obtained from different testing runs. To evaluate performance and quality control, the three different isolates of the ten most frequent *spa* types identified were re analyzed using ViiA 7 instrument (Life Technologies, Carlsbad, CA) with the Melt Detector HRM Master Mix including Syto9 as the fluorescent dye (Life Technologies, Carlsbad, CA), and on the Rotor-Gene Q (Qiagen, Hilden, Germany) in combination with the Type-It HRM Master Mix including EvaGreen as the fluorescent dye (Qiagen, Hilden, Germany). On both instruments the settings for HRM were used as recommended by the manufacturers. Briefly, on the ViiA 7 instrument PCR conditions were a 10 min activation step at 95°C, 40 cycles 95°C for 10 seconds and 60°C for 1minute. HRM was executed at condition of 60°C to 95°C with a ramp rate of 0.025°C/second. On the Rotor-Gene Q PCR conditions were 10 minutes' activation at 94°C, 42 cycles 94°C for 7 seconds, 64°C for 30 seconds and a final step at 95°C for 1 minute and cooling to 40°C for 1 minute. HRM was performed from 61°C to 87°C with a ramp rate of 0.02°C/second and 100 reads/seconds. The sequences obtained were edited using Chromas software (version 1.45, Australia). The guidelines from the Ridom Spa Server database **(http://www.spaserver.ridom.de)** were used to assign the edited sequences to particular spa types according to **Mayerhofer *et al.*, 2015**.

3.11. Evolutionary relationship of MRSA among humans and animals

To investigate diversity and infer transmission event, frequencies were compared among MRSA spa types isolates. Demonstration of MRSA isolates exhibiting the same spa-type in humans exposed to cattle or Swines and respective family members was regarded as transfer. Transmission of MRSA was assessed by demonstration of isolates exhibiting the same spa-type among family member or domestic animals. Nucleotide sequences of spa types (and spa repeats) were obtained from Ridom SpaServer (http://www.ridom.de/spaserver/). Maximum parsimony tree based on spa gene of all isolates of MRSA was created representing spa strains with accession numbers to arrange the phylogenetic tree based on specimens and spa types, and repeats. Using the Based Upon Repeat Pattern (BURP) algorithm within Ridom StaphType software (http://www3.ridom.de/spa-server/; version 1.5.17; Ridom GmbH, Wurzburg, Germany), DNA sequence repeats of *spa* types were downloaded and the dataset prepared into FASTA format. Phylogenetic analysis following DNA sequences in FASTA format were aligned using forward and reverse sequences of all amplicons using *Muscle W* in the BioEdit and MEGAX software. For phylogenetic tree construction and determination of the phylogenetic relationships of MRSA isolates based on the spa gene data, the sequences and the evolutionary history inferred using the maximum parsimony method **(Sokal *et al.*, 1973)**. The optimal tree with the sum of branch length = 0.32853604 is shown in figure 01. The evolutionary distances were computed as unit number of base substitutions per site using the Maximum Composite Likelihood method (**Tamura *et al.*, 2004**). The analysis involved 27 nucleotide sequences and Codon positions included were 1st+2nd+3rd+Noncoding (**figure 02**). However, all locations comprising of gaps and omitted data were removed. There were a total of 47 positions in the final dataset. Evolutionary analyses were conducted in MEGA X **(Kumar et al., 2016)**.

3.12. Data processing and analysis

Data processing and analysis included collection of data from the research administered questionnaires and data abstraction forms, processing, entry followed by analysis by SPSS. Research administered questionnaires and data abstraction forms were used to collect data from the respondents and the laboratory respectively. The questionnaires were designed in such a way for the respondent to answer in a format that met the research specific objectives and later be used to collect data. Questionnaire were tested for fitness to ensure accuracy and reliability followed by pre testing before it is used. Data abstraction form were designed and used to provide information generated from the laboratory work in relation to the set research specific objectives.

In order for data generated to provide meaningful information, it was cleaned using textual spell checker to lessen the amount of mistyped words, followed by data organization, then coded prior to entry into SPSS for analysis. Data processing involve validation, sorting, summarization and aggregation to ensure that the data generated is clean, correct and useful for easy of entry. Generated data was analyzed using SPSS, to give descriptive statistics which included frequencies, percentiles and ratios.

3.13. Quality control

Validity of the data was tested to ensure that questions administered conforms to the conceptual frame work of the study following **Amin (2003)** recommended validity index of at least 0.7. Valid laboratory data was ensured through running control strains following clinical and laboratory standard institute **(CLSI, 2017)** guidelines. The reliable laboratory results were achieved through use of quality reagents and instruments from reliable suppliers as well as use of control strain of bacteria. MSA quality was determined using control strains, S. aureus ATCC 25923 and ATCC 43300. Every new batch of Blood Agar Plates (BAP) and Mueller Hinton agar (MHA) were quality controlled using control strains included *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. Quality control

ranges for antibiotics were determined routinely for every new batch according to CLSI and QC form filled (Appendices 2,3 and 4)

3.14. Ethical considerations

Ethical approval was obtained from Faculty Research and Ethics Committee of Mbarara University Science and Technology and Institutional ethics and research committee (IERC). The informed consent was obtained from patients, community members and owners of respective domestic livestock. The collection of theses samples from the subject were not invasive with full respect of Human and animal rights following ethical guidelines (**Perlman *et al.*, 2010**).

CHAPTER FOUR: RESULTS

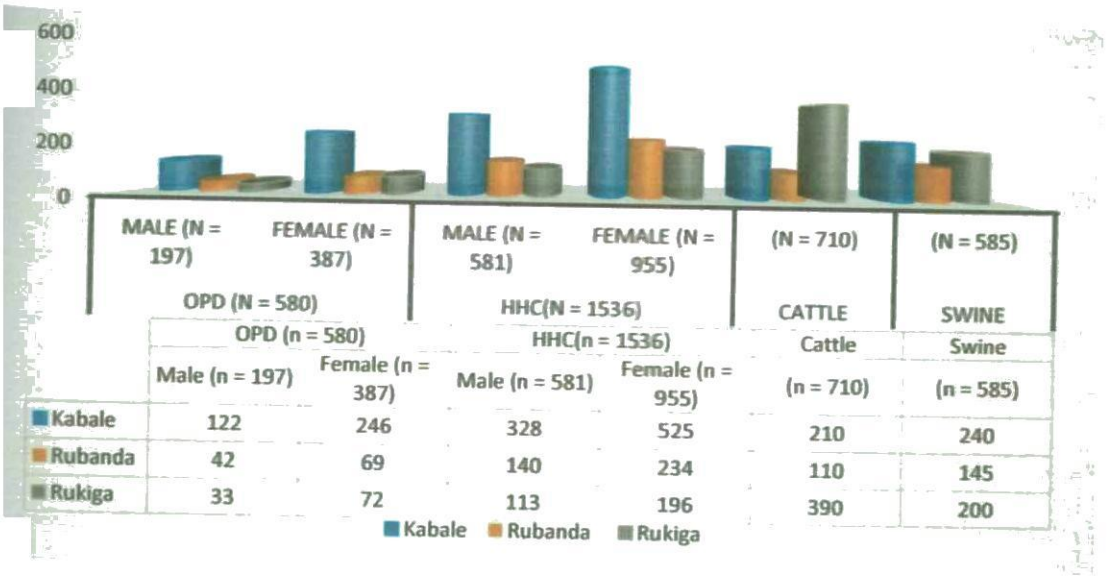
4.1.Introduction

The findings of this study resulted from the methodologies used to answer research questions stated in chapter 1 section 1.6, that seek to determine nasopharyngeal carriage rate of MRSA among outpatients at Kabale Regional Referral Hospital (KRRH) and their respective household human (HHC) and livestock (cattle and swine) contacts in greater Kabale, the prevalence of MRSA among inpatients admitted at KRRH and establishing the differences among MRSA strains isolated among livestock (swine and cattle) and Human. Additionally, the results are presented using statistical tables and graphics with narratives.

4.2.Demographics of outpatient and household contacts (Human and livestock)

The study enrolled 580 outpatients (OP) with 34% (n = 197) males and 66% (n = 387) females. Participants were from districts of Kabale; 63%(n =368), Rubanda; 19% (n = 111) and Rukiga; 17% (n = 101). The HHC enrolled were 1536, among which 38%(n = 581) were males and 62% (n = 955) females. These came from Kabale; 56% (n = 853), Rubanda; 24% (n = 374) and Rukiga; 20% (n = 304).

The livestock animals enrolled for the study included cattle (n = 710) and swine (n = 585). The cattle hailed from the districts of Kabale; 29.5%(n = 210), Rubanda; 15.5%(n = 110) and Rukiga 55%(n = 390). In addition, the swine came from the districts of Kabale; 41%(n = 240), Rubanda; 25%(n = 145) and Rukiga; 34%(n = 200).



	OPD (n = 580)		HHC(n = 1536)		Cattle	Swine
	Male (n = 197)	Female (n = 387)	Male (n = 581)	Female (n = 955)	(n = 710)	(n = 585)
Kabale	122	246	328	525	210	240
Rubanda	42	69	140	234	110	145
Rukiga	33	72	113	196	390	200

Figure 08. Demographics of outpatients and household contacts enrolled for the study

4.3. Nasopharyngeal carriage of S. aureus and MRSA in the outpatients at KRRH

We collected 580 swabs from females (n= 387) and males (n = 193), among which 300(51.7%) had *S. aureus* resulting into Nasopharyngeal colonization to be 51.7% in general outpatients (Table 1). 135(69.9%) males and 165(42.6%) female participants had *S. aureus* colonization. Among the *S. aureus* (n= 300) isolated, 104(17.9%) was MRSA and 196(33.7%) was MSSA. Therefore, the MRSA Nasopharyngeal colonization in outpatients attending KRRH was17.9%. However, 71(18.3%) females had MRSA compared to 33(17.7%) males. Majority of the *S. aureus* isolated were MSSA 196(33.7%) with the males (102; 52.8%) having highest MSSA colonization compared to females (94; 24.3%).

Table 1. Status of Nasopharyngeal colonization by S. aureus in Outpatients

Sex	*Nasopharyngeal swabs (n=580)*	*S. aureus (n= 300)*	*MRSA (n = 104)*	*MSSA (n =196)*
Males	193(33.3%)	135(69.9%)	33(17.7%)	102(52.8%)
Females	387(66.7%)	165(42.6%)	71(18.3%)	94(24.3%)
n (%)	580(100%)	300(51.7%)	104(17.9%)	196(33.7%)

p-value 0.05

Foot note: n = total number; MRSA= Methicillin resistance Staphylococcus aureus; MSSA= Methicillin sensitive Staphylococcus aureus.

4.4. *S. aureus* and MRSA Isolated from the Household human contact in greater Kabale region

We collected1536 swabs from females (n= 955) and males (n = 581), among which 799(52%) had *S. aureus* resulting into Nasopharyngeal colonization to be 52% in the community (Table 1). The colonization prevalence among men (n = 581), was 366(63%) and in females (n = 955) was 392(41%). Among the *S. aureus* (n= 799) isolated, 160(20%) was MRSA and 639 (80%) was MSSA, consequently, the prevalence of MRSA Nasopharyngeal colonization in in the community (n =1536) was n= 160; 10.4%. However, within females (n = 955) and males (n = 366), the MRSA prevalence was 20% (n= 191) and 19 %;(n = 70) respectively.

Table 2.Status of nasopharyngeal colonization by S.aureus (MRSA and MSSA) in household contacts human

	S.aureus; 52%(n = 799)	**MRSA; 10.4% (n= 160)**
Males (n = 581)	366(63%)	70(19%)
Females (n = 955)	392(41%)	90(23%)

pv =0.05

Foot note: n = total number; MRSA= Methicillin resistance S. aureus.

4.5. Nasopharyngeal carriage rate of MRSA among health care workers

A total of 120 Health care workers (HCWs) were recruited during the study of which 60 were staff nurses, 40 Clinicians and specialist doctors, 05 laboratory technicians and 15 were ward support staff. Of the 120 HCWs, 46 (38.3%) had shown *S. aureus* colonization of the nasopharynx, among which 13 (10.8%) were MRSA. Nurses (n = 60) had *S. aureus* colonization; 18(30%), Clinicians and other specialists (n = 40); 13(30%), ward support staff (n = 15); 15(100%) and Laboratory techs (n = 05); 01(20%). MRSA colonization among Nurses were 06(10%), Clinicians and other specialists; 05(11%), Ward Support staff; (02(13%) as shown in table 03.

Table 3. MRSA among Staphylococcus aureus isolated from Health care workers

Health care workers(HCWs) (n = 120)	***S.aureus* (n = 46)**	**MRSA(n = 13)**
Nurses (n = 60)	18(30%)	06 (10%)
Clinicians and other specialists (n = 40)	12 (30%)	05 (11%)
Ward Support staff (n = 15)	15 (100%)	02 (13%)
Laboratory techs(n = 05)	01 (20%)	0

4.6. *S. aureus* and MRSA isolated from inpatients admitted at KRRH

Four hundred and fifty (450) admitted patient's specimens were enrolled for the study. The specimens were collected from pediatric wards (n = 46), Obstetrics ward (n =115), surgical wards (n = 226), Medical wards (n = 50), Gynecology ward (n = 5) and psychiatric unit (n = 8). These clinical specimens were collected without repetition from inpatients in the period of May 2015 to January 2017 as part of routine clinical work. *MRSA = Methicillin resistance S. aureus; MSSA = Methicillin sensitive S. aureus.* Among these clinical specimens (n =450), there was pus swabs collected from surgical sites (*n=308*), bed sore wounds (*n =10*), burns wounds (*n = 20*), Abscess (*n = 7*) and RTA wounds (*n=33*). Other samples were Catheter-associated urine (*n = 5*), blood (*n = 12*), sputum (*n = 30*), Urine (*n =20*) and joint aspirates (*n=5*). All the specimens were processed and cultured as previously described where 390(86.7%) *S. aureus* isolates were realized.

Table 4. Staphylococcus aureus profile isolated from various clinical specimens of KRRH

Type of infection	***Specimen (n = 450)***	***S.aureus***	***(n = 390)***	***MRSA***	***(n = 223)***
Surgical site	308	292(94.8%)		166(56.9%)	
Bed sore	10	07(70.0%)		05(71.4%)	
Catheter-associated UTI	05	04(80.0%)		02(50.0%)	
Abscess	07	06(85.7%)		02(33.3%)	
Burn wound	20	16(80.0%)		09(56.3%)	
Bacteremia	12	09(75.0%)		04(44.4%)	
Pneumonia	30	11(36.7%)		08(72.7%)	
Osteomyelitis	05	03(60.0%)		02(66.7%)	
Urine	20	14(70.0%)		06(42.9%)	
RTA wounds	33	28(84.9%)		19(67.9%)	

Foot note: n= total number; UTI = urinary tract infection; RTA = Road traffic accident;

S. aureus, was predominantly isolated from specimens collected from surgical sites; 292(74.9%), RTA wounds; 28(7.2%) and burns wounds; 16(4.1%). Others were isolated from urine; 14(3.6%), sputum; 11(2.8%), blood; 09(2.3%), Bed sores; 07(1.8%), Abscess; 06(1.5%), catheter associated urine; 04(1.0%) and joint aspirates; 03(0.8%) as shown in the table 05. Among the *S. aureus* isolated, there was 223(57.2%) phenotypic MRSA and 167(42.2%) phenotypic MSSA. MRSA was significantly more frequently isolated compared to MSSA from all the infections (table 01). The MRSA isolated (n =223) was mainly from surgical site specimens; 166(74.4%), RTA wounds; 19(8.5%), Burn wounds; 09(4.0%); sputum; 08(3.6%), urine; 06(2.7%), Bed sore wounds; 05(2.2%), Blood; 04(1.8%). Others were isolated from the catheter associated urine; 02(0.9%), Abscess; 02(0.9%) and joint aspirates; 02(0.9%).

4.7.S. *aureus* and MRSA isolated from swine and cattle in Kabale district

A total of (n=254, 43.4%) of the 585 Swines sampled in the study were culture positive for *S. aureus.* The overall MRSA rate within the isolated *S. aureus* was n= 172; 29.4% and therefore, the prevalence of MRSA within the swine population was 29.4 %. In addition, a total of (n=283, 40%) of the 710 cattle sampled in the study were culture positive for *S. aureus.* The overall MRSA rate within the isolated *S. aureus* was n= 170; 23.9% and therefore, the prevalence of MRSA within the swine population was 23.9% see table 06.

Table 5. Staphylococcus aureus isolated among Cattle and Swine from Study homesteads of greater Kabale

Livestock	***S.aureus (n = 300)***	***MRSA(n = 104)***	***MSSA(n =196)***
Swine (n = 585)	254(43.4%)	172(29.4%)	82(14 %)
Cattle (n = 710)	283(40.0%)	170(23.9%)	113(16%)

Foot note: n= total number; MRSA = Methicillin resistance S. aureus; MSSA = Methicillin sensitive S. aureus.

4.8. Antibiotic susceptibility patterns of MRSA

4.8.1. Antibiotic resistance pattern of MRSA from Outpatients

A total of 580 nasopharyngeal swabs from the out patients, 300 (51.7%) *S. aureus* were isolated. MRSA were identified in 104(34.7%) of all *S. aureus* isolates or 17.9% (104) isolated from nasopharyngeal swab specimen (n = 580). All MRSA isolates (n = 104) were resistant to Amoxicillin - coadunate and cefixime while all the isolates were sensitive to vancomycin, linezolid and teicoplanin. However, other rates of resistance to Erythromycin, Clindamycin, Rifampin, cefotaxime, Gentamycin, Ciprofloxacin, Chloramphenicol, TMP-SMX, tetracycline and imipenem among MRSA were 93 (89%), 73 (70%), 24 (24.2%), 101 (97%), 51 (49%), 75(72%), 40(39%), 103(99%), 11(11%) and 30(29%) respectively. Among the MRSA, 84(81%) had phenotypic inducible clindamycin resistance(iCR) trait (Table 7).

4.8.2 Antibiotic resistance pattern of MRSA from Community house hold contacts

A total of 1536 nasopharyngeal swabs were collected from the community house hold contacts among which 799(52 %) *S. aureus* were isolated. MRSA were identified in 160(20%) of all *S. aureus* isolates or 10.4 % (160) isolated from nasopharyngeal swab specimen (n = 1536). All MRSA isolates (n = 160) were resistant to Augmentin, Cefotaxime, trimethoprim –sulfamethoxazole (TMP-SMX) and cefixime while all the isolates were sensitive to vancomycin, linezolid and teicoplanin. Other rates of resistance were seen against erythromycin, clindamycin, rifampin, gentamycin, ciprofloxacin, chloramphenicol, tetracycline and imipenem as 130 (81%), 120(75%), 50(31%), 65 (41%), 70 (44%), 40(25%), 117(73%), 40(25%) and respectively. Among the MRSA, 50(31%) had phenotypic iCR trait (Table 7).

4.8.3 Antibiotic resistance pattern of MRSA from Health care workers at KRRH

Among 120 nasopharyngeal swabs from the Health care workers (HCWs), 46 (38.3%) S. aureus were isolated. MRSA were identified in 13(28.3%) of all S. aureus isolates or 28.3% (13) isolated from nasopharyngeal swab specimen (n = 120). All MRSA isolates (n = 13)

were resistant to sulfamethoxazole/trimethoprim, cefixime, cefotaxime and Amoxicillin – clavunate while all the isolates were sensitive to vancomycin and teicoplanin. However, other rates of resistance to erythromycin, clindamycin, rifampin, gentamycin, ciprofloxacin, chloramphenicol, tetracycline, imipenem, linezolid, and teicoplanin among MRSA were 12 (92%), 10 (77%), 04 (31%) and 10 (77%), 11 (85%), 12(92%), 10(77%), 07(54%) and 01(8%) respectively. There was 04 (31%) MRSA isolates that had iCR phenotypic trait (Table 7).

4.8.4 Antibiotic resistance pattern of MRSA from in patients at KRRH

Among 450 non repetitive clinical specimens, 390 (86.7%) *S. aureus* were isolated. MRSA were identified in 223(57.2%) of all S. aureus isolates or 49.6% (223) isolated from clinical specimen (n = 450). All MRSA isolates (n = 223) were resistant to sulfamethoxazole/trimethoprim and Augmentin while all the isolates were sensitive to vancomycin. Rates of resistance to cefixime, erythromycin, clindamycin, rifampin, cefotaxime, gentamycin, ciprofloxacin, chloramphenicol, tetracycline, imipenem, linezolid, and teicoplanin among MRSA were 218(98%), 218 (98%), 207(93%), 134 (60%), 199(89%), 176(79%), 156(70%), 207(70%), 98(44%), 05(2.24%) and (12)5.38% respectively. Of note, 93(42%) 0f the MRSA isolates had phenotypic inducible clindamycin (iCR) trait Table 7)

4.8.5 Antibiotic resistance pattern of MRSA from in Swine

Among 585 nasopharyngeal swabs from the swine population, 254(43.4%) S. aureus were isolated and MRSA were identified in 172(67.7%) of all S. aureus isolates or 29.4% of the total swine non-repetitive nasopharyngeal swab specimen (n = 585). All MRSA isolates (n = 172) were resistant to Augmentin and Sulfamethoxazole/trimethoprim while all the isolates were sensitive to teicoplanin. However, other rates of resistance to cefixime, erythromycin, clindamycin, rifampin, cefixime, gentamycin, ciprofloxacin, chloramphenicol, tetracycline, imipenem, vancomycin and Linezolid among MRSA isolates were; 40 (23%), 153 (89%), 120 (70%), 40 (23%), 167(97%), 84(49%), 124(72%), 120(70%), 170(99%),26(15%), 03

(2%) and 03(2%) respectively (Table 7). There was 10 (6%) Among the MRSA isolates that had induced phenotypic induced clindamycin resistance trait. (Table 7).

4.8.6 Antibiotic resistance pattern of MRSA from Cattle

A total of 710 non-repetitive nasopharyngeal swabs collected, 283(39.9%) *S. aureus* were isolated, among which 170 (60%) MRSA were identified in 283 of all S. aureus isolates (23.9% of MRSA in Cattle population). All the MRSA isolates; 172 (100%) were resistant to Augmentin and Cefixime while 170 (100%) were sensitive to Imipenem, vancomycin, linezolid and Teicoplanin. Rates of resistance to erythromycin, clindamycin, rifampin, cefotaxime, gentamycin, ciprofloxacin, chloramphenicol, sulfamethoxazole/trimethoprim and tetracycline among MRSA were 116(68%), 70 (41%), 46 (27%), 153 (90%), 83(49%), 129(76%) 116(68%) 168(99%), and 163 (96%) respectively. Among the MRSA; 50 (29%) showed phenotypic iCR (Table 7).

Table 6. Antibiotic susceptibility patterns of MRSA isolates from Humans, Cattle and Swine in greater Kabale

Antibiotics	**In patients (n = 223)**	**Outpatient (n =108)**	**Community (n = 127)**	**Swine (n = 131)**	**Cattle (n = 137)**
Augmentin	223(100%)	108 (100%)	160(100%)	153(89%)	170(100%)
Cefixime (30µg)	218(98%)	108 (100%)	160(81%)	40(23%)	170(100%)
Erythromycin (15µg),	218(98%)	90 (87%)	130(94%)	153 (70%)	116(68%)
Clindamycin (2µg),	207(93%)	73 (70%)	120(75%)	120(70%)	70(41%)
Rifampicin (5µg),	134(60%)	24 (23%)	50(31%)	40 (23%)	46(27%)
Cefotaxime (30µg),	199(89%)	103 (97%)	100(63%)	167(97%)	153(90%)
Gentamicin (10µg)	176(79%)	51(49%)	65(41%)	84(49%)	83(49%)
Ciprofloxacin (5µg)	156(70%)	75 (72%)	70(44%)	124(72%)	129(76%)
Chloramphenicol	207 (70%)	40 (39%)	40(25%)	120(70%)	116(68%)
TMP-SMX (1.25/23.75µg)	223(100%)	103 (99%)	160(100%)	170(99%)	168(99%)
Tetracycline (10µg)	143(64%)	11(11%)	117(73%)	120 (70%)	163(96%)
Imipenem (30 µg)	98(44%)	30 (29%)	40(20%)	26 (15%)	00
Vancomycin (30µg)	02(0.9%)	00	00	03(2%)	00
Linezolid	05(2.24%)	00	00	03(1.7%)	00
Teicoplanin	12(5.38%)	00	00	00	00
iCR	93(42%)	84(81%)	50(31%)	10(6%)	50(29%)

Foot note: iCR = induced clindamycin resistance

P vale 0.05

4.9. Spa typing and phylogeny of MRSA

4.9.1. Circulating MRSA spa types in Humans, Cattle and Swine

A total of 2370 consented humans and 1472 animals were involved in the study, where among humans, outpatients(OPD) were 384(16.2%), inpatients (IP) 450(19.0%), House hold contacts 1536(64.8%). Among the animals, cattle were 1164(79.1%) and swine 308(26.4%). We isolated 726 MRSA isolates from human; 458(19.3%) distributed in OPD; 108(28.1%), HHC; 127(8.3%) and IP; 223(49.6%) while in animals; 276(18.2%), cattle and swine accounted for 137(11.8%) and 131(42.5%) as shown in table 01.

Table 7. Staphylococcus profile isolated from Humans and domestic cattle and swine of greater Kabale

Specimen source	Total	*S. aureus*	MRSA
	(n = 3842)	2084(54.2%)	726(18.9%)
Outpatient (OPD)	384	124(32.3%)	108(28.1%)
House hold contact (HHC)	1536	458(29.8%)	127(08.3%)
Inpatient(IP)	450	390(86.7%)	223(49.6%)
Cattle	1164	978(84.0%)	137(11.8%)
Swine	308	134(43.4%)	131(42.5%)

Footnote: n= sample size; MRSA = Methicillin resistant S. aureus. P>.005.

A total of 726 MRSA isolates were typed and twenty-seven (n = 27) *spa* types, including t1358 Cattle and Swine MRSA isolates. However, the different *spa* types were randomly distributed among the humans and animal subjects. It appears the whole MRSA spa types were not limited to specific humans or animals, where certain types could be identified from isolates whose origin The dominant *spa* types observed as tabulated in table 9 were; t701(CC6) 19.6%(142), t9377(CC45);68(9.4%), t1081(CC45);65(9.1%), t4677(CC398); 62(8.5%), t121(CC8);46(6.3%), t002(CC5); 40(5.5%) and t034(CC398) as well expounded in table 09. The *spa* types t1081, t701, t4677, t1451, t9377, t121, t002, t019, t108 and t018 were significantly associated with Humans (OPD and HHC) and IP and among Cattle and Swine. In addition, t044 were highly

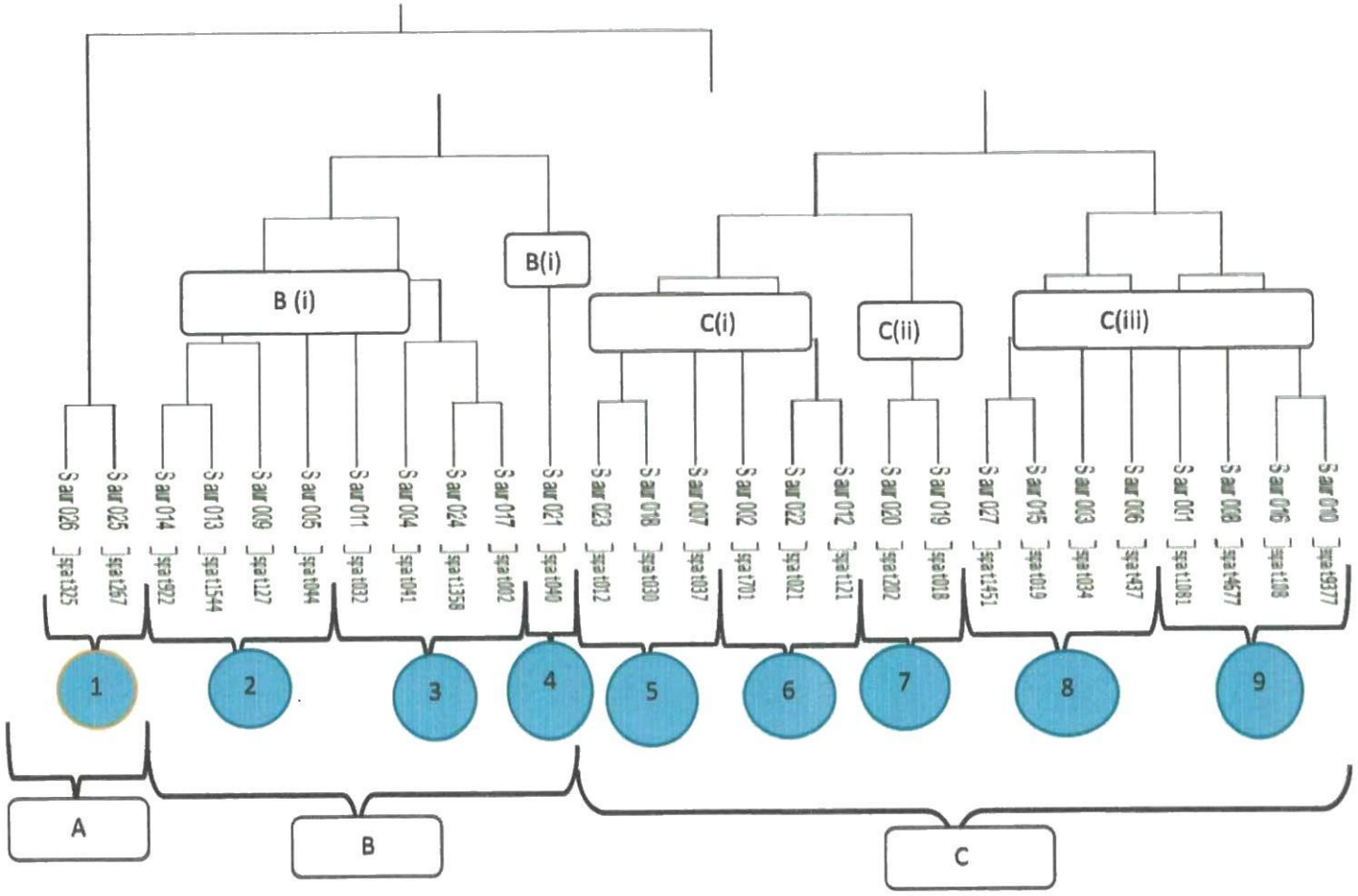
associated with OPD, HHC and IP. However, *spa* types t012 (CC30), t021(CC30), t012(CC30), t267(CC97), t325(CC78) and t1358(whose clonal complex could not be established), were only associated with hospitalized patients (IP). *Spa* types; t108(CC398), t1451(CC398) and t4677(CC398) and t002(CC5) and t041(CC5) which are known to be typical Livestock associated MRSA (LA MRSA) were identified in human population. However, among the cattle and swine, spa types; t034(CC398), t1081(CC45), t1451(CC398), t4677(CC398) and t108(CC398) were predominating. Spa type t922 was only identified from MRSA isolates whose origin was swine and outpatient while t1358 was only identified from MRSA isolated from inpatients. In addition, spa types t922 (CC1), t1544 (CC20) were only identified from MRSA isolates related to outpatients.

We note that, spa type's t127 and t922 belong to CC1 while t202 belong to CC3. CC5; t041 and t002, CC6; t707, CC45; t1081, t9377 and t040. CC398 had t034, t4677, t1451 and t108. CC80; t044, CC59; t437, CC8; t037 and t121, CC22; t032, CC20; t1544, CC30; t019, CC8/239; t030, CC3; 202, CC30; t021and t012, CC97; t267 while CC78; t325. The CC of t1358 was not established

Table 9. spa types and repeat sequences of MRSA isolates from Humans, Cattle and Swine of greater Kabale

spa types	Clonal complex (CC)	No. of isolates (n = 726)	Repeat of spa types	OPD (n = 108)	HHC (n = 127)	Cattle (n = 137)	Swine (n = 131)	IP (n = 223)
t1081	CC45	65(9.1%)	26-23-17-34-17-20-17-34-17-16	9	14	21	11	10
t701	CC6	142(19.6%)	11-10-21-17-34-24-34-22-25-25	24	25	1	3	12
t034	CC398	12(1.7%)	08-16-02-25-02-25-34-24-25	3	2	41	40	3
t041	CC5	11(1.5%)	26-30-17-34-17-20-17-34-17-20-17-12-17-16	4	5	2	0	0
t044	CC80	29(4.0%)	07-23-12-34-34-33-34	4	1	0	0	24
t437	CC59	14(1.9%)	04-20-17-20-17-25-34	6	3	3	2	0
t037	CC8	7(0.9%)	04-20-17-20-17-25-34	3	2	0	2	0
t4677	CC398	62(8.5%)	08-16-02-25-02-24-25	11	14	14	14	9
t127	CC1	7(0.9%)	07-23-21-16-34-33-13	2	5	0	0	0
t1451	CC398	11(1.5%)	08-16-02-25-34-25	1	3	22	19	5
t9377	CC45	68(9.4%)	08-16-16-02-43-34-17-34	7	15	2	1	5
t032	CC22	15(2.1%)	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	1	2	0	0	12
t121	CC8	46(6.3%)	11-19-21-17-34-24-34-22-25	12	9	9	11	5
t1544	CC20	1(0.1%)	07-22-34	1	0	0	0	0
t922	CC1	2(0.3%)	07-23-21-16-33-13	1	0	0	1	0
t019	CC30	36(5.0%)	08-16-02-16-02-25-17-24	2	6	11	9	8
t108	CC398	33(4.6%)	08-16-02-25-24-25	4	6	6	8	13
t002	CC5	40(5.5%)	26-23-17-34-17-20-17-12-17-16	6	9	3	4	17
t030	CC8/239	13(1.8%)	15-12-16-02-24-24	3	0	0	0	10
t018	CC30	21(2.9%)	15-12-16-02-16-02-25-17-24-24-24	4	3	2	2	10
t202	CC3	5(0.7%)	11-17-23-17-17-16-16-25	1	1	2	1	0
t040	CC45	3(0.4%)	09-02-16-13-17-34-16-34	0	1	1	1	0
t021	CC30	30(4.1%)	15-12-16-02-16-02-25-17-24	0	0	0	0	30
t012	CC30	10(1.4%)	15-12-16-02-16-02-25-17-24-24	0	0	0	0	10
t1358	New*	20(2.8%)	26-23-13-23-31-29-17-25-17-25-28	0	0	0	0	20
t267	CC97	10(1.4%)	07-23-12-21-17-34-34-34-33-34	0	0	0	0	10
t325	CC78	10(1.4%)	07-12-21-17-34-13-34-34-33-34	0	0	0	0	10

Figure 09. Evolutionary relationship of spa types of MRSA isolates from humans, Swine and cattle of greater Kabale



The phylogenetic trees indicate the ancestral history of the spa types within MRSA. The radiating branches of the phylogenetic tree indicate a different line of evolution from the original ancestor. Phylogenetic analysis of the spa gene sequences was performed using both the neighbour-joining and maximum-parsimony methods in the MEGA X software package. After several bootstrapping replications, the consensus trees derived were virtually identical and only the consensus tree by the maximum-parsimony method is shown (Figure 03). This is contrasted to the consensus tree derived by the neighbour-joining method from spa gene sequences (Fig. 2). Based on spa sequences, the 27 spa types identified within MRSA isolated can be grouped into nine major clusters; 1,2,3,4,5,6,7,8 and 9 (Figure 03). It appears group A which consists of highly related spa types; t267 and t325 diverged earlier from groups B and C forming their independent cluster 1. Overtime group B consisting of clusters; 2, 3 and 4 diverged earlier from group C which consists of clusters 5,6,7,8, and 9 from their common ancestor. However, within group C, several sub groups emerged; C(i) closely related to C(ii) while C(iii) closely related to C(iv). Spa types; t9377, t108, t4677 and t1081 are highly related to each other within cluster 9. Similarly, cluster 8 consisting of spa types; t437, t034, t019, t1451 are highly related to each other while cluster 7 consists only two closely related spa types t108 and t202. The cluster 6, there are t121 closely related to t021 in comparison to t701. Also, cluster 5 has spa types; t030 closely related to t012 but distant from t037. On the similar note, group B diverged into two subgroups (B1 and B2) from their common ancestor where B2 has only cluster 4 that consists of only one spa type; t040. However, B1 further evolved to form cluster 2 and 3. Cluster 2 had spa types; t922, t1544, t127 and t044 that are closely related while cluster 3 consists of closely related spa type; t002, t1358, t041 and t032.

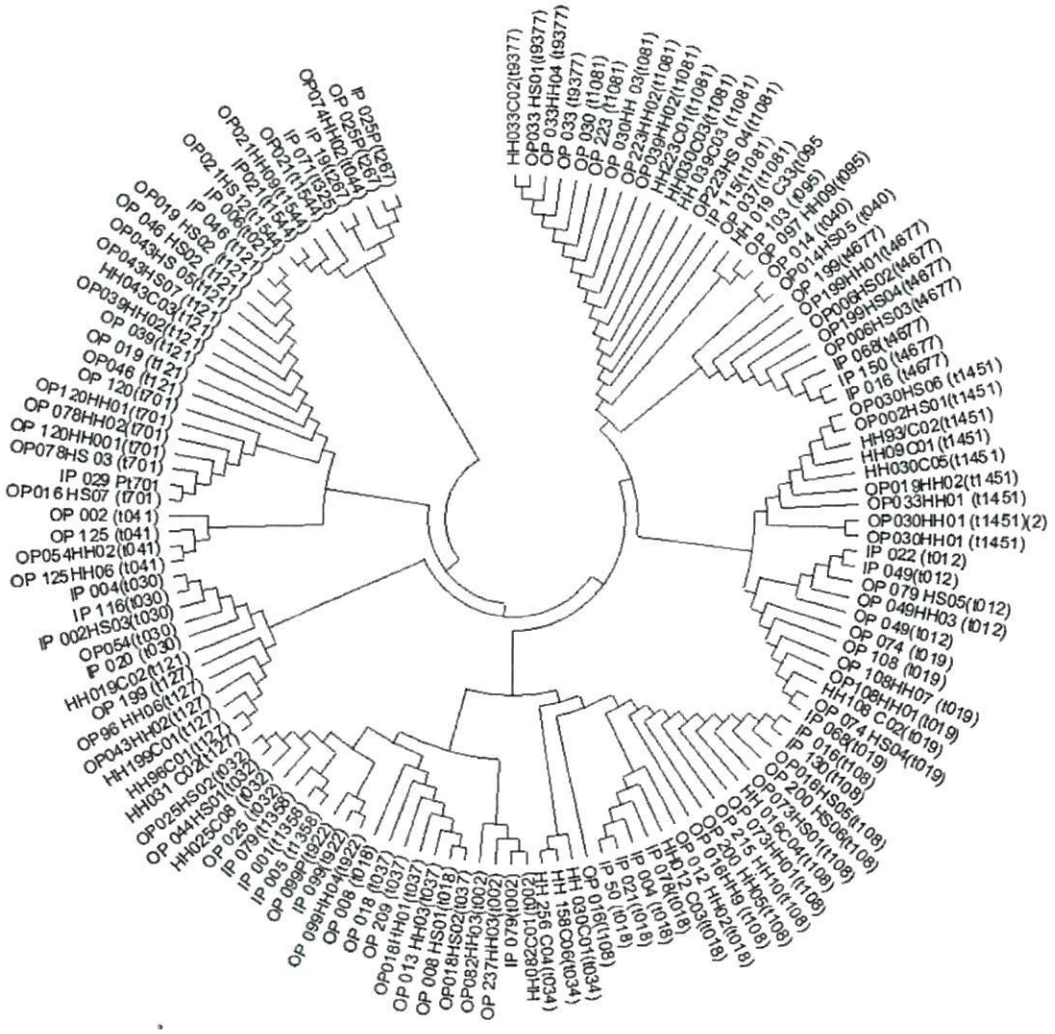
Analysis of the 27 spa types allowed identification of a pool sharing similar topologies. Rooting spa sequences reveals that two closely related t325 and t267 *spa* type which forms

clade 1 which was the first and oldest lineage that diverged independently and away from other *spa* types circulating within Humans and animals. They were only identified in the MRSA strains isolated from the hospitalized patients. The phylogenetic tree presented in **Figure 09** is a highly accurate depiction of the evolutionary relationships among the spa types in the MRSA isolates. The horizontal axis of the dendrogram represents the distance or dissimilarity between clusters; 1,2,3,4,5,6,7,8, and 9 where the rectangular boxes to the right indicates predicted clans. The most ancestral lineage (clade 1) was composed entirely of closely related MRSA strains, spa types; t325 (CC78) and t267(CC97) which branched off early from the rest of the family and were found to have originated from IP at KRRH. However, clades 2 to 9 though branched off from clade 1 and shared common ancestral origin evolved overtime to produce various clans of related spa types. Looking at the dendrogram (figure 02), the eight clusters as eight branches that occur at about the same horizontal distance depicting similar time in evolution. The two outliers, clade 7 (spa types t018- CC30 and t202 – CC3) and clad 4 (spa t040 -CC45), are fused in rather arbitrarily at much higher distances. We notice the clade 9 consists of monophyletic group of *spa* types; t9377(CC45), t108(CC398), t4677(CC398) with t1081(CC45) which evolved earlier but showing its common ancestry and are very similar. Overtime, clade 8 shared ancestral origin with clade 9, forming its own monophyletic group consisting of spa types; t437(CC59), t034(CC398), t019(CC30) and t1451(CC398). Originally, clades 7,6 and 5 are believed to have evolved from a common ancestor and all its lineal descendants resulted into distinct *spa* types in respective clad 7: t018(CC30, t202 (CC3), 6: t121(CC8), t021(CC30), t701(CC6) and 5:t037(CC8), t030 (CC8), t012(CC30). Evolutionary, clades 4,3 and 2 descended from common ancestry with clades 5 to 9 long ago. However, clad 4: t040(CC45) was emerged faster than clads 3; t002(CC5), t1358(New), t041(CC5), t032(CC22) and clad 2; t044(CC80) t127(CC1), t1544(CC20), t922(CC1) which evolved into distinct clans. There was no

paraphyletic species and therefore mother MRSA isolates that gave rise to distinct daughter spa types become extinct.

Figure 10. Rooted circular dendrogram clustering of MRSA isolates based on spa type profiles by maximum-parsimony

Evolutionary analyses were conducted in MEGA X. maximum-parsimony, rooted, circular dendrogram clustering of MRSA isolates based on spa type profiles. The spa types are from different sources; Human from outpatients (OPD), respective House hold contacts (HH), Inpatients (IP) and OPD respective domestic animals; cattle (HHC) and swine (OP HHS) as indicated respectively



The evolutionary history was inferred using the Maximum Parsimony (MP) method. The most parsimonious tree with length = 184 is shown in figure 10. The consistency index is 0.478261 (0.460674), the retention index is 0.938383 (0.938383), and the composite index is 0.448792 (0.432289) for all sites and parsimony-informative sites. The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 0 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 170 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 442 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. The whole circle indicates splits with >90% bootstrap support that contain all isolates from the current study, and are therefore deemed greater Kabale regional groups.

We compared the genetic relatedness of MRSA from human and MRSA isolated from cattle and swine using spa DNA nucleic typing. The phylogeny of MRSA spa sequences analyzed indicated dispersing spa strains among the study population with exceptions spa types t267 and t325 both isolated from inpatients(IP). Carriage isolates from Cattle and swine and those isolated from humans (HHC and OP) were highly related (Figure 02) suggesting continuous acquisitions between and among animals and humans within homesteads or onward transmission to others. A link between human and domestic animals subject is explained by multistep inter and intra transmission events. Human and animals in homesteads together where a presumed transmission event took place resulting into similar spa types circulation. For instance, Both IP025 and OP 025 are more closely related with Spa types t267 while house hold contact OP 074 HH02 is closely related but associated with spa type t044 and IP 19 and IP071 were more related to each other with spa type t267. From spa type t1544 was linked to swine OP021HS12 and humans (IP021, OP021HH09 and OP21) and were closely related.

In addition, IP 006 were closely related IP 046 and linked to spa type t121. These were also related to swine; OP019HS02, OP046HS02, OP043HS05, OP043HS07, cattle; HH043C03, household contact; OP039HH02 and outpatients; OP 039, OP 019 and OP 046. In close note, spa type t701 is traced in inpatient; IP 029 and close to swine OP016HS07. It is originated from outpatient; OP 120 and respective house hold contact; OP120HH01, OP078HH02 and swine; OP078HS03. Close to t701, is spa type t041 which is traced from outpatients OP002 and OP 125 and house hold contacts OP054HH 02 and OP125HH06.

Also, MRSA spa type t030 was traced in MRSA isolated from inpatient IP 020 and moved to, then to swine; OP 002HS03, inpatients; IP 116 and IP 004.However, spa t127 which is closely related to spa type t030 was seen in outpatients; OP 199 and respective household contact; OP96HH06 and OP043HH02 and cattle; HH199C01, HH96C01 and HH031C02.

Among the MRSA isolates, there was spa type t032 which was traced in swine; OP025HS02, OP043HS01 and cattle; HH025CO8 which are linked to outpatient; OP 025. They are closely related to inpatient IP 079, IP 001 and IP 005 which has spa type t1358, that is more close to spa type t922 which is identified in OP099, IP 099 and house hold contact OP099HH04.

Among spa type t037 was traced in swine; OP018HS02 and OP008HS01, House hold contact; OP013HH03 and OP018HH01 up to outpatients; OP 209, OP018 and OP008. However, spa type t002 was linked to inpatient IP 079 closely related to cattle HH082C01. This was also close to household contact OP237HH03 and OP082HH03.

Of note, spa type t108 was linked to IP016 which is close to IP 130 and related to swine; OP016HS05, OP016HS06, OP200HS06 and OP73HS01, cattle; OP016C04 and house hold contacts; OP073HH01, OP215HH10, OP200HH05, OP016HH9 and OP012HH02. A closely related spa type t018 was only linked from inpatients; IP50, IP02, IP004 and IP 078 which was closely linked to cattle; HH012C03 and house hold contact; OP12HH02.

We also observed spa type t019 linked to inpatients; IP068 and swine; OP074HS04 and cattle; HH108C02 which was closely related to house hold contact; OP108HH01 and OP108HH07 as well as OP 108 and OP 074. In addition, outpatient; OP 049 and house hold contact; OP049HH03 as well as swine; OP079HSO5 were linked to spa type t012 which was also be linked to inpatients; IP049 and IP022. But, a closely related spa type t1451 was only linked to household contacts; OP030HH01, OP033HH01, OP019HH02 and cattle; HH030C05, HH09C01, HH93C02 and swine; OP002HS01 and OP030HS06.

Also, among the outpatients, IP0156, IP150 and IP 068 were linked to spa type t1451 and related to swine; OP006HS03, OP199HS04, OP006HS02 and OP199HS01 and to outpatient; OP199. This was close to spa type t040 which was only found in outpatient; OP014 and respective domestic swine; OP14HS05.

Outpatient; OP0103, household contact; OP097HH09 and cattle HH019C33 was linked to spa type t095 while outpatients; OP037, OP 030 and OP223 inpatient; IP115, house hold contacts; OP223HH02 and OP039HH02, Cattle; HH030C03 and HH039C03 as well as swine OP223HS04 were linked to spa type t1081 while spa type t9377 was associated with outpatient; OP033 closely associated with respective household contact; OP033HH04 and domestic cattle; HH033C02 and swine; OP033HS01.

CHAPTER FIVE: DISCUSSION

5.1.Introduction

Precisely, there no studies that has previously been performed in greater Kabale region to investigated nasopharyngeal carriage frequency of MRSA in human, respective livestock as well as antibiotic resistance and genetic diversity of MRSA. In an effort to fill this knowledge gap, we phenotypically and genotypically characterized MRSA isolates from OP, HHC, IP (from different wards at KRRH) and livestock animals (cattle and swine) in the greater Kabale region. We designated methodologies to test all study specimens with utmost quality control. Despite the fact that MRSA in Uganda has been described, the limited data in rural setting of greater Kabale region is now demystified in reference to the aim of the current study, which was to determine the prevalence, spread and genetic relationship of MRSA isolated among humans (community, inpatients and Health care workers) and domestic cattle and swine in Kabale District - western Uganda. The four sub-aims - the first to determine the nasopharyngeal carriage rate of MRSA among the outpatients and health care workers at KRRH, determine the prevalence of MRSA among the *S. aureus* isolated from inpatients admitted at KRRH, To establish the presence of MRSA among domestic cattle and swine owned by those individuals found to have MRSA at the OPD of KRRH, and to establish the relatedness of MRSA strains isolated from inpatients, Health care workers, outpatients and their respective House hold contacts (Community) and domestic animals swine and cattle. These aspects were described in the previous chapter that presented the methodology used adhering on the study design.

Based on our research findings, we confirmed the presence of MRSA in all subjects, there is eminent relationship among MRSA strains that exists among humans and animals. Therefore, Chapter five provides a discussion of the main findings from the current research and where applicable, links the literature to the research outcomes.

5.2.Nasopharyngeal carriage of *S. aureus* and MRSA in the outpatients at KRRH

The first research objective investigated the nasopharyngeal carriage rate of *S. aureus* and MRSA among the outpatients. The findings provide significant prevalence of MRSA colonization and insights on antimicrobial resistance rates. The study showed *S. aureus* colonization rates among males was 135(69.9%) and significantly higher in females165(42.6%); ($p<0.05$) attending outpatient department of KRRH, with average prevalence of 51.7%. This is in agreement with the prevalence observed in Gulu, Uganda where S.aureus prevalence was 50% in males and 46.7% in females (**Kitara et al., 2011**). This study is the first to report on the prevalence of *S. aureus* at OPD of KRRH. However, previous study by Kitara et al reported slightly low prevalence of 48.3% among outpatients in 2011 at Lacor Hospital in Northern Uganda (**Kitara et al., 2011**) while in United States of America, John et al reported a much lower prevalence of 24.7% among patients attending outpatient clinics in 2015(**John et al., 2003**). Geographical difference probably may contribute to these discrepancies in addition to improved infection, prevention and control in developed countries as compared to rural setting of greater Kabale region. Various risk factors like smoking, alcoholism and hygiene probably explains the differences in colonization rates. Sangvik et al, reported similar findings in Norway (**Sangvik *et al.*, 2011**). We suggest further subtype screening to demonstrate age cluster and gender association among the human populations as well as factors associated with this phenomenon. In general, the *S. aureus* carriage rate among outpatients was 51.7% which is much higher than what was reported in several studies elsewhere overtime **(Chatterjee *et al.*, 2009)(Stefani *et al.*, 2012)(Huang *et al.*, 2006)(Song *et al.*, 2011)**. This signifies geographical and population variations in terms of *S. aureus* colonization. The high prevalence could be attributed to poor hygiene **(Dryden, 2009)**, household crowding or immunosuppression (**Uluğ *et al.*, 2011)(Dryden, 2009)**. People colonized with *S. aureus* were at increased risk of MRSA

colonization. A significant number of outpatients (104(17.9%) were colonized with MRSA at the time of outpatient visit. The study did not establish if this was the first or second visit to the hospital. However, this signifies and urgent need for screening for MRSA among this population, an important task necessary for hospital infection prevention and control. Our findings are much more higher than what have been reported previously in India; 5% **(Sharma *et al.*, 2014)** and United states; 0.84% **(Graham et al.,2006)**, while Khanam et al reported high prevalence of 23.29% in Bangladesh **(Khanam *et al.*, 2017)**. Differences in the prevalence of Nasopharyngeal carriage of *S. aureus* strains (MSSA and MRSA) between geographical locations and health care facilities may be explained in part by differences in the quality and size of samples, the use of different microbiological methods (from sampling technique to culture media) and probable differences in the interpretation guidelines. In addition, diverse levels of obligation to infection control processes may yield these differences. No previous similar study has been carried out in the same region and therefore we state the likely existing risk to the health care facility (**Mest et al., 1994**).

Of particular concern, we observed increasing resistance to antimicrobial agents among MRSA compare to MSSA. Our findings suggest high and multiple-drug resistant characteristics in the MRSA strains. The susceptibility test results showed Augmentin and Cefixime to be not effective agent with resistance rate of 100% which is in sharp contrast to the resistance rates of 72% stated by Saeide et al in Iran **(Saeide et al., 2014).** Antibiotic sensitivity pattern of the MRSA isolates (Table 07) shows 100% sensitivity to Vancomycin, Linezolid and Teicoplanin. These findings are consistent with a study from Korea and United states **(Choo et al., 2016)** where 100% MRSA were resistant to 100% sensitivity towards Imipenem, Linezolid and Vancomycin, but slightly disparity from what has been reported in Nairobi, Kenya **(Gitau et al,. 2018)** with sensitivity to Imipenem being 98% , vancomycin; 95% and Linezolid; 95% . All the MRSA isolates from the outpatients were multidrug

resistant in the present study. It is alarming to note that 72% isolates from OPD were resistant to ciprofloxacin since it had been suggested to be a substitute for treatment of MRSA skin infections (**Smith et al., 1989**). Increasing number of MRSA resistant strains may be the result of selection pressure owing to uncontrolled drug usage in the community or probable multiple hospital visits.

According to Kaka et al, trimethoprim/sulfamethoxazole, antibiotic have been in use for several decades showing excellent activity against *S. aureus*, including MRSA (**Kaka *et al.*, 2006**). However, in our current study, the resistance rate was 103(99.0%) which is consistent with 82.3% reported from India (**Kali *et al.*, 2013**) and 68% reported from Iran (**Shokouhi *et al.,2017)***. However, this is higher than 63.2% (**Rajaduraipandi *et al.*, 2006)** and 42.7% (**Alvarez-Uria *et al.*, 2012**) reported in India reported in India. TMP/SMX use as prophylaxis in patients with HIV has been associated with the development of TMP/SMX-resistant flora, including *S. aureus* (**Wood *et al.*, 2012**). It has been suggested that exposure to other antimicrobials may also contribute to TMP/SMX resistance through selection for plasmids containing multiple antibiotic resistance genes (**Ito *et al.*, 2009**). Screening of patients before use can control spread of resistant strains

The increasing MRSA resistance to erythromycin and clindamycin represents a gruesome situation where antibiotics usage is reduced further (**Mwambi et al., 2014**). For instance, this study revealed that 93 (89.4%) of MRSA were erythromycin resistant while 73 (70.2%) were resistant to clindamycin. Unlike beta lactam antibiotics, both macrolide and lincosamide antibiotic agent inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells and resistance to these antimicrobial agents occur through bacterial methylation ribosomal target site resulting into macrolide-lincosamide-streptogramin B resistance (**da Paz Pereira *et al.*, 2016)(Wilson, 2014**). In addition, 84 (81%) of MRSA had iCR and this is much more higher compared to similar studies elsewhere (**Ujwol *et al.*, 2016)(Katakweba *et***

al.*, 2016)**. erythromycin resistant *S.aureus* isolates maybe susceptible to clindamycin but can still be resistant to clindamycin by an inducible or constitutive mechanism encoded by the *erm* gene by methylation of the 23S rRNA (**Ferreira *et al.*, 2015)(Sida *et al.*,2015**). This phenomenon is possible due to the fact that Erythromycin is an effective inducer, yet clindamycin is a weak inducer (**Drinkovic *et al.*,2001**) and therefore, clinical failure may arise when clindamycin therapy is instituted on patients who previously been exposed to erythromycin. Ciprofloxacin is a fluoroquinolone commonly prescribed in this region, this study has indicated raising MRSA resistance of 75(72%). This high MRSA resistance has been reported previously elsewhere (**Chakrakodi et al., 2014)(Beeton et al.,2015)(Ojulong, *et al.*, 2009*) and therefore, its clinical usefulness remains questionable because of this raising resistance, yet, quinolones, particularly ciprofloxacin, could be the best alternative to vancomycin due to its ease of administration and low toxicity. We are concerned with this phenomenon envisaging continuous narrowing of treatment choices for the management of common MRSA infections.

Currently, Aminoglycosides in particular, gentamycin still play an important role in management of *S.aureus* infections in this region (**Chatterjee *et al.*, 2009**), however, according to the current study, the emerging resistance amongst MRSA has been observed (49%) which is consistent with what 48% reported from Tehran, Iran (**Rahimi, 2016**). However, Neeta et al, reported 26% MRSA resistance to gentamycin which is much lower that what we reported (**Neeta et al., 2014**). Gentamycin is among the commonly used antibiotic in both injectable form and eye drops and this can explain the raising resistance observed.

Low levels of Rifampicin resistance (23%) were observed, probably due to limited use in the public as the antibiotic is reserved for treatment of tuberculosis (**Health, 2010**). As an adjunctive therapy, Rifampicin is an antibiotic of substantial interest in the rise of MRSA

infections (**Li *et al.*, 2016**), however, the disclosed resistance in this current study is an alarm to remind clinicians that its usage is limited. Of note, MRSA presenting from the community is sometimes associated with previous silent acquisition in the healthcare environment, or household contacts (**Gould et al., 2009**). Once established within hospitals settings any MRSA is difficult to control and its survival is probably promoted by the increasing use of antibiotics. Selection of new clones of MRSA may follow changes made in usage in antibiotic prophylaxis and treatment. The time course for evolution and spread of an antibiotic-resistant strain probably depends on the antibiotic in use (**Wood *et al.*, 2012**).

5.3. Nasopharyngeal carriage of *S. aureus* and MRSA among Household contacts from in greater Kabale region

While infections with MRSA were traditionally restricted to the hospital setting, novel MRSA strains emerged over the last two decades that have the capacity to infect otherwise healthy people outside of the hospital setting. These MRSA strains combine methicillin resistance with enhanced virulence and fitness. By far the most frequent disease manifestation associated with CA-MRSA is infection of the skin and soft tissues (**Fridkin *et al.*, 2005**).

S.aureus associated with Household contacts is an evolving cause of skin and soft-tissue infections in several parts of the world (**DeLeo *et al.*, 2010**). Recently, many cases of MRSA infection have been reported in healthy community individuals with no traditional risk factors for MRSA infection (**Wood *et al.*, 2012**). *S. aureus* nasopharyngeal carriage rate in the present study was 52% with MRSA nasopharyngeal carriage prevalence of 10.4%. This is consistent with the overall nasopharyngeal MRSA carriage rate of 10.4% reported from United states (**Wood *et al.*, 2012**). However, Sharma et al found a prevalence of 58.33% of *S.aureus* among the population in Delhi, India which is a higher than observed in our current study (**Sharma *et al.*, 2014**). Amongst MRSA strains reported is in agreement with Cirkovic

et al who reported nasopharyngeal carriage rate of MRSA to be 11.8% in in Bulkans (**Cirkovic *et al.*, 2015**). A study performed in the urban area of Bangalore reported MRSA nasopharyngeal carriage rate of 22.5% among the healthy individuals in the communities (**Alvarez *et al.*, 2012**). This probably suggests the difference in MRSA prevalence among rural and urban settings and population density. The results of current study indicate that high prevalence of nasopharyngeal carriage of MRSA found in greater Kabale region, indicating MRSA could probably be replacing MSSA. This shifting trend where MRSA is replacing MSSA colonization is a major threat to the public health. However, more studies are needed in other parts of Uganda to accept these findings.

Among the females, the MRSA carriage rate was 17.7% compared to males (18.3%) and was not statistically significant (Table 2). This contradicts Sharma et al, who reported males to be the major carriers of MRSA (**Sharma, et al., 2014**). The spread and increasing prevalence of MRSA in the community likely can spill over into the hospital settings via outpatients and such can have important implication for hospital infection control programs. Methicillin resistance is often associated with resistant to the most widely used class of *antibiotics*, called beta-lactams, which include penicillin, methicillin and carbapenems worrisomely other classes of antibiotics (**Alvarez-Uria et al., 2012**). In the present study, MRSA isolated among household contacts had higher levels of resistance to Augmentin, erythromycin, Cefixime, and trimethoprim – sulfamethoxazole. Increasing availability of over the counter antibiotics and self-medication coupled with poor antibiotic stewardship could be the probable cause of this phenomenon (**Kaur *et al.*, 2015**). It is important to consider presence of multi drug resistance among MRSA isolated from household contacts before initiating antibiotic prescriptions. Empirical prescription of antibiotics against *S. aureus* infections is encouraged by the lack of microbiology testing laboratories to identify and perform antibiotic susceptibility testing to help in clinical judgment. Agreeing with the current results, it appears

beta-lactam antibiotics are no longer useful to treat *S. aureus* infections. However, antibiotic resistance patterns of MRSA isolated from community vary considerably in different parts of the world (**Kong et al., 2010**). Therefore, knowledge of antibiotic susceptibilities of MRSA is highly required before any commencement of antibiotic treatment in the region greater Kabale region and elsewhere. There was no resistance to Vancomycin, Linezolid and Teicoplanin antibiotics. This is in agreement with Shivaprakasha who reported low levels of MRSA resistant to vancomycin, linezolid, and Teicoplanin in Mumbai, India (**Shivaprakasha, 2015**). We also found that majority of MRSA strains isolated from the communities were susceptible to Imipenem (80%) and chloramphenicol (75%) and these antibiotics could probably be used when high risk factors for MRSA infections are anticipated. One of the limitation of this study was inability to determine the presence of Panton-Valentine leukocidin (PVL) gene. Studies have reported presence of *pvl* gene that carry PVL virulence factor in the majority of MRSA strains from the community (**Cirkovic *et al.*, 2015**). The high prevalence of MRSA in the community suggest an urgent need to strengthen infection control programs in both rural communities and health care settings of greater Kabale region and elsewhere. Community hygiene and coupled with health education can minimize MRSA nasopharyngeal colonization (**Olawale et al., 2011**).

5.4.*S. aureus* and MRSA isolated from the inpatients admitted at KRRH

Our findings suggest that *S.aureus* prevalence (86.7%) was high among inpatients at KRRH than previously reported in other Ugandan hospitals (**Lubega et al., 2017**)(**Anguzu et al., 2007**) and elsewhere (**Jean-Marie et al., 2014**)(**Schaumburg *et al.*, 2014**). The findings confirm results of earlier studies demonstrating that infections due to *S. aureus* are among the highest common bacterial infection in hospital setting (**Khan *et al.*, 2017**). In Mulago National Hospital, Seni et al reported prevalence of *S. aureus* and MRSA to be 20.4% and 37.5% respectively (**Seni *et al.*, 2013**) which is lower than we report today. However, this

reflects an impeding threat to the hospitalized patients in both health care settings. This calls for strengthening of infection control practices in KRRH and elsewhere. S *aureus*, frequently cause infection in hospitalized patients including infants and is associated with increased mortality, morbidity, and length of hospital stay **(Phu *et al.*, 2016)**. Infection prevention strategies should broadly be enhanced to target *S. aureus* in this population. Postoperative surgical site infections remains a significant cause of morbidity in surgically treated patients on addition to high cost due to prolonged stay in hospitals and need for advanced management and drugs **(Kim *et al.*, 2014)**. Surgical and obstetric wards where surgery is the predominating mode of treatment has increased *S.aureus* isolates (94.8%) more than previously reported in other studies **(Lubega *et l.*, 2017) (Anguzu et al., 2007)(Ranjan *et al.*, 2017)**. These findings reflect inadequate postoperative care and failure to practice aseptic surgical procedures as well as poor infection control and overcrowding of hospital facility **(Baguma *et al.*, 2016)(Sharma *et al.*, 2014)**. *S. aureus* is a particularly complex, virulent, and successful pathogen and the increasing incidence of *S.aureus* bacteremia; 75%(n = 09) absurdly maybe associated with advancements in medicine that requires frequent use of more invasive technologies like catheters and implanted devices **(Souza et al., 2014)(Naber, 2009)**. However, low levels of *S.aureus* bacteremia was reported to be low as previously reported in other health facilities **(Kumalo et al., 2016) (Mugalu et al., 2014)**, therefore this current study reveals an increased cases of sepsis. We must also note that many of patients seek self-medication locally with antibiotics and this affects bacterial recovery from blood culture **(Christoph *et al.*, 2009)**. Infections of open musculoskeletal injuries due to road traffic accident (RTA), continue to pose a challenge in surgical patients, where *S. aureus* continue to be the earliest bacterial contaminants of these open wounds. 84.9% (n = 28) S.aureus indicates the burden this bacteria pose to this group of patients **(Kumalo *et al.*, 2016)**. The burn wound is among other major health problem at KRRH and in the present

study, *S. aureus* was 80% which is much more lower compared to the previous studies **(Bagdonas et al., 2003)**. However, open and large areas of burn injuries create favorable conditions for the penetration of hospital infection and much attention be paid in ward hosting burns patients **(Alebachew et al., 2012)**. There was high *S.aureus* prevalence of 72%, probably due to long-term stay of care patients or which is in agreement with previous studies elsewhere **(Muder *et al.*, 2006)**. Of much concern today there is the emergence of MRSA, including strains with reduced susceptibility to antibiotics and increased virulence (Figure 1&2). In addition, our results confirm significant high rates of MRSA (49.6%) among the *S. aureus* isolates. MRSA among inpatients have been described before and their association with multiple antibiotic resistance patterns **(Baguma et al. 2016)(Lubega *et al.*, 2017)(Bagdonas *et al.*, 2003)**. Certainly, Chakrakodi *et al* **(Chakrakodi *et al.*, 2014)** reported much higher rates of MRSA (59%) compared to current study, an indication of rising worldwide threat of MRSA. However, it is important to note that, the current study was on patients with potential predisposing risk factors to healthcare-associated MRSA infections, including previous surgeries, severe burns, road traffic accident trauma, the presence of indwelling devices such as catheters and lengthy hospital stays. There were no significant differences among males and females in terms of gender predilection. Invasive surgical procedures are always the main mode of treatment especially at Surgical wards and obstetric wards **(Galukande *et al.*, 2010)**. However, postoperative surgical site infections with *S.aureus* remains a significant cause of morbidity in surgically treated patients with prolonged hospitals stays requiring additional cost of care that directly affects the patients and hospital settings **(C.J. Kim *et al.*, 2014)**. *S.aureus* infections to the surgical sites at both Surgical and obstetric wards is alarming high than previously reported in other studies **(Lubega *et al.*, 2017)(Anguzu et al., 2007)(Ranjan *et al.*, 2013)**. Our findings expose the manifestation of inadequacies and failure to uphold strict sterility during surgical procedures

while paying less attention during postoperative attention. The inadequate infection control and poor hygiene coupled with resource and structural constraints grossly contributes to spread of bacterial infections (**Solís-Téllez *et al.*, 2017**). We propose that awareness regarding hospital acquired infections among healthcare workers and the general population should be over emphasised feasibly through routine health education and promotions. *S. aureus* and MRSA presence in urine was common among those patients with urinary catheter devices. The presence of a urinary catheter is the most important risk factor for bacteriuria which adversely affect patient outcomes. Catheter devices can serve as a focus for haematogenous spread leading to sever bacteraemia and persistent Catheter related bacterial infections **(Souza *et al.*, 2014)(Naber, 2009)**. However, we identified low levels of blood borne *S. aureus* isolates than previously reported elsewhere **(Kumalo *et al.*, 2016)(Mugalu *et al.*, 2012)**. Low yield could probably due to previous antibiotic therapy before blood culture is taken. It has been shown that previous antibiotics therapy decreases bacterial recovery from blood cultures**(Grace *et al.*, 2001)(Christoph, 2009)**.

S. aureus, (both MRSA and MSSA) are of concern in their ability to cause difficult to heal infection of the musculoskeletal injuries or wounds. Wounds are a risk factor for colonization by pathogenic bacteria, including multidrug-resistant microorganisms like *S. aureus* particularly MRSA which interferes with wound healing with consequent increase in the severity of the lesions. At present, the overall prevalence of *S. aureus* infection of burn wounds was high (80%) with majority of the isolates being MRSA. This is much higher than previously reported by Alebachew et al in Ethiopia (**Alebachew *et al.*, 2012)(Bhat *et al.*, 2010**).However, *S. aureus*, was found to be the predominating organism affecting burn wounds in Ethiopia (69.5%) **(Alebachew *et al.*, 2012)** and in Ghana (60%) **(Amissah *et al.*, 2017**).Colonisation of burn wounds with microorganisms especially from normal resident flora in skin is almost certain to occur in patients with severe burns and it is crucial to

investigate the profile of these microorganism like *S. aureus* as such would increasingly disseminate MRSA in hospitals. For every 10 cases of bed sores (pressure ulcers), seven are infected with *S. aureus.* Bed sores are more strongly associated with prolonged hospital stays **(Yoshikawa et al., 2002)**. The incidence of pressure ulcers in a hospitalized patient is often a direct measure of the quality of medical care provided, particularly in the meticulous attention paid to careful positioning and frequent turning of the bedridden patient. We observed that 85% of all RTA were colonized by *S. aureus*. This is in agreement with the study in Malaysia where Wong et al reported *S.aureus* as the most prevalent bacteria found in wounds (**Wong, *et al.*, 2015).** Over 58% of the wounds colonized by *S. aureus* were colonized by MRSA, which is similar to previously reported findings **(Stryjewski *et al.*, 2014)(Luna et al., 2010) (Bindu D, 2017)**.

This present study found MRSA prevalence of 49.6% with high rate of antibiotic resistance. This is higher than is previously reported in S. Korea (**Kim *et al.*, 2014**) Australia **(Sadsad et al., 2013**) and India (**Paterson et al., 2014**). This may be attributed to prior antibiotic misuse by patients, the majority of whom were elderly with comorbidities, and use of multiple antibiotics. In addition, previous hospitalization is among the contributing factor that may have resulted in selection of MRSA. Presence of MRSA in hospitalized patients indicates a need for greater control to ensure the proper use of antibiotics, and careful inpatient management and his environ within the hospital. The higher prevalence of MRSA observed inpatients admitted in this hospital reflects the burden of antimicrobial resistance in resource constrained health care settings. MRSA bacteria are likely to be more pathogenic and the infections may not respond to treatment especially to beta-lactam antibiotics. The virulence and pathogenicity of MRSA (fig 1&2) probably due to presence of high virulent strains among the isolates from this region remain a subject desired to be studied.

High prevalence of MRSA in inpatients have been described before **(Murthy *et al.*, 2010)(Senok *et al.*, 2016)** and their association with antimicrobial resistance to multiple antibiotics **(Kejela and Bacha, 2013)(Lowy, 2003). Chakrakodi *et al*** (**Chakrakodi *et al.*, 2014**) detected a 59% of MRSA with multi antibiotic resistance from inpatients admitted in a tertiary care hospital in Bengaluru, South India. This is much higher than the rate found in our study and could be explained by a difference in study population. The presence of multidrug-resistant MRSA at KRRH highlights a poorly addressed issue. Among the MRSA; there was 100% resistance to TMP - SMX, and Amoxicillin – clavunate. Our findings were consistent with a previous report from Democratic Republic of Congo (DRC) in which MRSA collected from different clinical specimens showed TMP - SMX resistance prevalence of 100%. However, did give low Amoxicillin – clavunate resistance prevalence of 89% **(Lyamba *et al.*, 2014)**. The increase of MDR - MRSA prevalence could be explained not only by the long use of these antibiotics, but also by an infection or concurrent colonization with other resistant bacteria **(Harmon *et al.*, 2010) (Costa *et al.*, 2015)**. The widespread use of antimicrobial agents has unquestionably augmented the development of resistant *S. aureus,* acquiring multiple resistance genes and therefore capable of surviving in almost all antibiotic families, i.e., beta – lactams, Tetracycline, fluoroquinolones **(Weber *et al.*, 2003)**(Couderc *et al.*, 2014)., Macrolides (Blanco *et al.*, 2016) and aminoglycosides **(Rahimi, 2016)(Khosravi *et al.*, 2017)**. In the present study we demonstrated a great resistance rate to gentamycin in MRSA strains tested was 71.7%, which is higher than previously reported from Iran (47.8%)**(Rahimi, 2016)** and lower than (84.5%) according to Khosravi et al. *(***Khosravi *et al.*, 2017)**. Gentamicin in particular, is widely used in KRRH to treat a variety of bacterial infections, including serious bacterial infections. Similarly, we are reporting a resistance rate of 80.7% for Tetracycline which is higher than similar reports from Tehran (61%) **(Emaneini *et al.*, 2013)**. Tetracycline is a broad-spectrum antibiotic that have been widely used and

therefore not surprisingly that tetracycline resistance is highly prevalent. We recommend characterization of the resistance determinants in the isolates since this group of antibiotics can be resisted by three different genotypes.

In addition, we observed that MRSA resistance to erythromycin, and was 70%. This consistence with the published report of Kaur *et al.*(**Kaur *et al.*,2015)** and Gandra *et al.*(**Gandra *et al.*, 2016**) who indicated that MRSA resistance to various antibiotics including erythromycin was higher than 70%. erythromycin resistance has increased dramatically which could due to unrestricted use this antibiotic in the region. In addition, MRSA isolates was also reported to be more than 50% resistant to ciprofloxacin and clindamycin. Weber *et al* also reported similar pattern of resistance by MRSA isolates to ciprofloxacin and other fluoroquinolones to be more than 50% (**Weber *et al.*,2003**). However, Mario *et al* had report much high resistance to ciprofloxacin among MRSA isolates from the hospital setting **(Weber *et al.*, 2003).**

Clindamycin has been an exceptional antibiotic *Staphylococcal* infections and an alternative in penicillin-allergic patients. Its desirable oral bioavailability has been credited as an excellent option for outpatient treatment and changeover after intravenous antibiotics **(Asim, *et al.*, 2017**). We report the prevalence rate of Erythromycin resistance as, 74.1%. This much higher than 39.4% reported by Mokta et al reported in India (**Mokta *et al.*, 2015**). The rate of inducible clindamycin resistance (iCR) (16.2%). is much lower than what have been previously reported elsewhere **(Asim *et al.*, 2017),(Ghosh *et al.*, 2016).** The consequence of excessive use of macrolides is responsible for resistance to these drugs. However, differences in the resistance rate among regions emphasize the need for generating a local resistance data to guide empirical therapy. Detection of iCR is important for the use of clindamycin in Staphylococcal infections including MRSA.

There is limited literatures regarding VRSA from clinical isolates in Uganda. The current study report 1.3% resistance rate of MRSA to Vancomycin. Our findings are probably among the first of the kind to be reported. These isolates were confirmed for the presence of vanA/B gene using Biofire FilmArray. The vanA gene that mediates vancomycin resistance in *S. aureus* is believed to be transferred from *Enterococcus faecalis* and *E. faecium* **(Reygaert, 2013)**. Finding VRSA isolates in a rural setting represents a serious public health concern. We suggest stringent infection control policies to prevent transmission of such life-threatening isolates in the hospital setting.

There was no MRSA isolates resistance against teicoplanin (Glycopeptides) and Linezolid observed. Walkey et al recommended empirical use of these antibiotics because of their presumed efficacy against MRSA (**Walkey, *et al.*, 2011**). However, we suggest that such decision should be based routine and regular antimicrobial surveillance, local availability, and local cost. High MRSA rates from inpatients may be due to a high prevalence in their community where they come from probably reflecting a long-term stable carriage(Sharma *et al.*, 2014b)(David and Daum, 2010), or they can be due to transmission between community and livestock (**Cuny *et al.*, 2015**). However, the diversity and origins of these organisms cannot be speculated unless typing is done.

5.5. MRSA colonization in domesticated Cattle and Swine.

Much as Methicillin Resistant *S. aureus* (MRSA) is increasingly becoming importance in hospitals (**Gould *et al.*, 2012**) and the community (**Vandenesch *et al.*, 2003**) as HA – MRSA and CA – MRSA respectively, the trend is seems similar to the livestock farming (**Ye *et al.*, 2016**). The present study provides novel information about *S. aureus* carriage rates among healthy Swines and cattle in greater Kabale region - south western Uganda. We observed *S. aureus* colonization rate of 43.4 % (n = *254*) and 40%(n = 283) in swine and cattle respectively. This considerable high percentage of *S. aureus* colonization in domesticated

animals that live in close proximity with humans is particularly a public health concern. In view of *S. aureus* colonization, this prevalence is consistent with various studies which demonstrated colonization of this pathogen in animals **(Christiane et al., 2013)(Schaumburg *et al.*, 2013)**. This current study did not elucidate the exact source of *S. aureus* to animals but we predict probable cross contamination among between humans and animals. Garipcin et al indicated that cattle could probably be the source of colonisation capitulated an urgent need for awareness, appropriate intervention and control measures to prevent the spread between animal and human populations **(Garipcin et al., 2015)**. In addition, we report MRSA prevalence of 23.9% (n = 270) and 29.4%(n = 172) among cattle and swine respectively. This consistent with 28.57% that was reported by Kumar et al from India **(A. Kumar et al., 2017)**. Presence of MRSA strains among S. *aureus* colonizing domestic animals is alarming since most of these strains are highly resistant to other antibiotics (Kilic *et al.*, 2006). However, the current study showed higher prevalence of MRSA than 2.95% MRSA in Swines and 1.6% MRSA in Cattle previously reported in Switzerland **(Koller et al., 2010)**. Lower prevalence of MRSA cattle have been reported in Nigeria (12.1%)**(Isa et al., 2016)** and Tanzania (4.4%) in Tanzania **(Jibril et al., 2018)**. This shows geographical variation in MRSA prevalence. Possible sources MRSA strains in *S. aureus* colonizing host could be acquisition of mecA gene from coagulase negative staphylococci belonging to the normal microflora of Livestock, which may have transmitted the mecA gene to a methicillin susceptible S. aureus strain. Alternatively, the MRSA strain as such may have been transmitted to these animals from another source possibly contaminated feeds or dust **(Pantosti, 2012)**. In household contacts humans may infect companion animals with MRSA and vice versa. A similar phenomenon may have been the initial cause of the emergence of the MRSA in domestic animals **(Neeling et al., 2007)**.

We examined the MRSA antimicrobial susceptibility patterns towards the commonly used human antibiotics in this region. MRSA isolates revealed a high number of multi-resistant strains highlighting the limited therapeutic options to treat any MRSA infections (Table 01). All MRSA isolates were resistant to antibiotics like penicillin G and amoxicillin which is in agreement with (**Crombé *et al.*, 2013**). Alarmingly, there was 1.9% (n = 03) isolates swine that were resistant to vancomycin. The presence of MRSA strains resisting vancomycin is perceived as a formidable threat in the therapeutic field revealing the necessity for new and effective drugs against MRSA. The abrupt appearance of vancomycin strains could probably due to continued increase in the use this antimicrobial agent in the community(Joshi and Devkota, 2014). Resistances to trimethoprim – sulfamethoxazole, gentamycin and ciprofloxacin have been reported worldwide (**Cuny et al., 2015**)(**Price *et al.*, 2012**) among livestock animal. Resistance to Resistances to trimethoprim – sulfamethoxazole was reported high in MRSA isolates (99%), but very low among MSSA isolates investigated in this study. This explains the possibility of multidrug resistance nature of MRSA(Edwards *et al.*, 2013)(**Vaz-Moreira et al., 2015**). Multidrug-resistant MRSA infections pose a significant threat to human health (**Edwards *et al.*, 2013**). The elevated frequency of trimethoprim – sulfamethoxazole and ciprofloxacin resistance found in this study may be related to the frequent use of this antimicrobial in animals (Price *et al.*, 2012)(Neela *et al.*, 2009)(**Faccioli-martins, 2011**). Antimicrobial use in Swines will select for LA-MRSA (**Broens 2015**)(Stefani *et al.*, 2012), and through direct and indirect contacts transmission within and between herds will occur. Subsequently, transfer to humans is likely through direct contacts, environmental or airborne transmission (**Cuny *et al.*, 2015**).

5.6.Genetic relatedness of MRSA isolates

MRSA strains in Kabale region are genetically diverse with an elevated prevalence of t701(CC6), t9377(CC45) and t1081(CC45), all significantly associated with hospital settings.

The polymorphic region X of the *spa* gene consists of a variable number of tandem repeats, generally 24 bp but repeats of 3 and 16 bp in length have been identified. The sequence variation in this region arises from duplications or deletions of repeats or point mutations within the repeat sequence resulting into various spa types. The, mutational rate in this region is relatively low, allowing for adequate discrimination between MRSA strains (**Martin et al., 2014**). There was high overlap of *spa* types among different sources (humans and animals) especially, *spa*-t1081, t701 and t034, t4677, t9377, t121, t019, t108, t018 and t002 was high. We observed the population structure of MRSA in Kabale region as remarkably diverse with over 27 spa types. The considerable diversity has been previously noted in other studies **(Cirkovic et al., 2015)(Moodley et al., 2006).** However, a study conducted in East part of Uganda agrees with the current on the diversity of circulating spa type but disagrees on the spa types identified which were; t002, t037, t064, t4353 and t12939 (**Kateete et al., 2019).** It is likely therefore that the *spa* type lineage studied in Kabale region has been established in this region for a considerable length of time. Evaluation of the MRSA *spa* types among humans at OPD and HHC with that from their respective domestic animals (Cattle and swine) displayed a large overlap spa types within both subjects. Multiple clades circulating in communities, are likely to have resulted from introductions from the wider population of t325 and t267 with subsequent local transmission to community and respective domestic animals (**Yaser et al., 2017**). The genetic relatedness among colonizing spa types suggests anthroponotic and zoonotic transmissions. The phylogenetic signal generated by the all *spa* types from humans and animals suggested bidirectional acquisition and transmissions resulting into diversity observed in this study. However, other studies maybe required to determine the validity of this observation. The phylogenetic signal generated by the all spa types from humans and animals suggested horizontally acquisition of nucleotide polymorphisms in each derived clade and is a highly accurate depiction of the evolutionary

relationships among the spa types in the MRSA isolates. *S. aureus* (including MRSA) is a commensal and major pathogen of humans and animals. Different spa types among MRSA populations perhaps suggests colonization of different host species associated with carriage of mobile genetic elements (MGE), principally bacteriophages and plasmids **(Fabio *et al.*, 2017**. Although, these are capable of encoding virulence, resistance, and immune evasion pathways, may probably transfer other spa genes horizontally resulting into spa diversity observed in our study.

Several lineages of *S. aureus*, including MRSA, are found in livestock species and can be acquired by humans through contact with animals and vice versa. The emergence of livestock-associated MRSA (LA-MRSA) isolates raises public health concerns because of the potentiality of livestock to act as reservoirs for MRSA outside the hospital setting. The *spa* types with CC398 and CC5 lineages detected are particularly of concern to the public health community because, unlike the isolates in the CC398 lineages, isolates in the CC5 lineage are a significant cause of human disease in both the hospital and community settings globally (**Hau *et al.*, 2017**). Colonization with MRSA belonging to CC398 (t108, t4677, t034 and t1451) were observed freely circulating in humans and animals. Nasopharyngeal carriage of MRSA CC398 and infection in humans has also been reported by previous studies (**Pantosti, 2012**). These MRSA belonging to CC398 could have been acquired through direct or indirect contact of humans with domestic animals (Swine and Cattle) (**Fitzgerald, 2012**). According to the data, measures for prevention of infections in humans by MRSA should focus on humans with direct exposure to livestock. We observed a dynamic change of distinct MRSA spa types clades among humans and animals over time, each of which had its own distinctive clade pattern. It was notable that each clade carried various spa types that are freely shared by IP, OP, HC and domestic animal subjects. This clearly indicates inter transmission events of MRSA. Thus, the findings are in agreement with commentary of

Fitzgerald 2012, and in the systematic review of **Klous *et al.*, 2016**. Comparison of the extent of the cloud with other isolates from humans and animals revealed an overlap, suggesting a common origin for these isolates and that extensive intra and inter transmission occurring in greater Kabale region. This reveals an important justification to further identify transmission events in community and clinical setting to gain an accurate understanding of transmission networks. A large proportion of spa types with hospital origins (IP) were found in clades IV and V. These are oldest clades and probably appears to have been a major source of inter transmissions. The transmission dynamics of MRSA between humans and animals were closely related. Our results are consistent with recent studies by **Crombé et al. 2013** and by **Sharma et al. 2016** who observed that colonization and infection, were a result of recent intra-human and animal transmission events.

We observed the role of household MRSA transmission has been recognized as a driver for the community epidemic, resulting in increased introductions of MRSA into healthcare facilities. Certainly, household transmission of t9377, t108, t4677, t701 could in part explain the multiple introductions observed in our study. The *spa* typing results of MRSA strains collected humans at OPD, HHC and hospital (IP) Cattle and Swine subgroups showed that eight distinct *spa* type linages, t1081, t701, t4677, t977, t019, t108, t121, t002 and t018, were significantly associated with the human and animal subgroups. Worrisome though, these strains have been identified in hospitalized patients elsewhere (**Cheng *et al.*, 2013**). This phenomenon indicates the potential role of OPD and IP and domestic animal transmission dynamics of these MRSA genotypes. Infection control should be focused on these points (at OPD and at discharge points when patients are leaving the hospital in order to reduce the burden of MRSA carriage. Our findings are consistent with a scenario in which diverse *spa*-type strains are imported from external reservoirs and subsequently lead to intra-hospital transmission events involving multiple patients (**Ferreira *et al.*, 2011**) (**Cheng *et al.*, 2013**).

The proportion of t325(CC78) and t267(CC97) that are closely related and found only in Clade V suggests that a significantly less diverse oldest population that exists within hospital settings. In study by **Havaei *et al.*, 2014**, these *spa* types (t325 and t627) were found once in hospital isolates. Evolutionary, MRSA – spa types t325 and t267 originated from hospitalized patients. Splitting into distinct strains of MRSA exist in the community and Livestock. Our findings are consistent with **Harrison *et al.*, 2016** who observed high rates of MRSA carriage and on-going MRSA transmission between hospitalized patients, and acquisition and onward transmission of MRSA during hospital admission. The environment and human to human contact as well as animal contact might represent an important MRSA transmission pathway. The OPD spa types sharing closely related isolates with in patients (IP) as shown in table 10, confirms potential onward transmission. We identified several spa types in IP related to domestic animals, which may be attributable to carriage of these lineages by OP or staff. Taken together, this suggests that the Humans who visit hospitals may be an important source for MRSA transmission and a barrier to effective infection control is urgently needed including cleaning to reduce MRSA contamination and infection rates in hospitals. This transmission network demonstrates the complex inter-connectivity of community – hospital setting.

5.7.Limitation of the study

We acknowledge several study limitations. HRM was used to generate spa Sequences of MRSA isolates. Furthermore, only the nares of cattle and swine were sampled in the homesteads in greater Kabale region, thus excluding MRSA carriers solely colonized at other body sites. In addition, due to difficult in funding, MRSA isolates from the healthcare workers were not typed, limiting assessment of their potential role in MRSA transmission.

The Population was larger and more diverse groups (humans and domestic animals) which over stretched the budget; however, this was solved by sticking to stated exclusion and

inclusion criteria. Another important challenge was determining the scope of the review which was considered during reviewing the available literatures especially those written based on observational studies. Increase in the budget allowed access to every qualifying subject in remote villages. We did not evaluate the presence of Panton-Valentine leukocidin (PVL) gene and whole genome sequences to discriminate nucleotide differences and allow accurate characterization of transmission, predict old and emerging strains as well as determining basis for phenotypic AMR and virulence.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1.CONCLUSION

The current study identified a high occurrence of MRSA strains in isolated *S. aureus* that is a significant threat to the public health in greater Kabale region as this bacterium can disseminate in community with consequent spill over into the clinical setting. This high prevalence of MRSA demonstrates a glaring need for periodic monitoring to fathom and describe MRSA infections. Maintenance of proper hygiene by in humans at both community and hospital level and avoidance to irrational use of antibiotics coupled with antibiotic stewardship could effectively reduce the rate and dissemination of MRSA. Good hygiene practices, both in the household and in human and animal healthcare environments is the primary strategy for prevention against MRSA spread and dissemination. *S. aureus* nasopharyngeal carriage amount the outpatient in the current study is 51.7% among which 17.9% are MRSA. Nasopharyngeal carriage rate of MRSA among healthy household contacts were significantly lower than the outpatients. However, health education, population screening followed by decolonization of carriers are probable effective prevention strategies to control the spread and burden of MRSA within the community of greater Kabale region.

The prevalence of S. *aureus* isolated from inpatients admitted at KRRH was high with alarming increasing MRSA prevalence (49.5%). This is definitely a significant threat with urgent need to reinforce an awareness campaign in the health care setting to adequately undertake universal precaution like routine hand washing and hygiene thereby minimizing the risk of transmitting MRSA and contamination.

The prevalence of S. *aureus* nasopharyngeal colonization among the cattle is estimated to be higher (40%) and the prevalence of MRSA is also recorded to be high (23.9%). In addition, the prevalence of nasopharyngeal colonization of *S. aureus* among the Swines was also higher (43.4%) with the MRSA carriage rate of 29.4%.

We report high diversity of spa types among MRSA circulating among the human and livestock (cattle and swine) of greater Kabale region. Inpatients had extra and unique spa – types; t021, t012, t1358, t267 and t325 that are not found in the community and livestock. the. This data can be used for epidemiological purposes, such as defining the origins of spread of the principal spa types of MRSA, frequencies, comparing the revolutionaries of MRSA lineages, and understanding transmission dynamics.

Relatedness of MRSA strains isolated from IPs, OPDs and respective HHCs and their respective animals (swine and cattle) around Kabale region was high and all species share similar strains of MRSA. At the same time, the majority of human MRSA isolates are directly associated with livestock and vice versa. Therefore, the origin of spread remains unknown. However, there is ongoing bidirectional transmissions of MRSA among human and livestock (anthroponotic and zoonotic transmissions).

MRSA strains are highly resistant to commonly used antibiotics in Kabale region. However, antibiotics, particularly, Imipenem, teicoplanin, linezolid and vancomycin were still effective against MRSA. MRSA strains with phenotypic constitutive and inducible clindamycin resistance is increasing becoming higher in both humans particularly in among inpatients, and livestock.

We are cconcerned about MRSA in both human and livestock and as these bacteria becomes fully established these hosts (man and his livestock), we predict impossible elimination. However, proper study of MRSA in livestock, characterization of intra and interspecies transmission, while identifying true human and livestock strains with their health risks, and improvement of evidence-based control measures is necessary to reduce the impact human and veterinary health.

6.2. RECOMMENDATION

This study shows that there is mutual MRSA transmission between humans and livestock. Therefore, surveillance at the interface of human and domestic is necessary. This should also pay consideration to the ongoing spread of MRSA strains between human and animals and to the emergence of human associated MRSA in domestic animals. The establishment and implementation of infection, prevention and control guidelines on MRSA is urgently needed.

Large community-based studies, molecular distinction between hospital acquired-MRSA and community acquired-MRSA, continuous surveillance, screening of target population, and decolonization of carriers should be conducted to decrease the spread and burden of drug resistant S. aureus and MRSA in the elementary schools and the community at large.

These findings support the need for more effective infection control measures to reduce carriage in whatever form and prevent dissemination MRSA in Kabale region and elsewhere. Infection control measures should be enhanced in all units of hospital settings. Information from this study may be used in future as a baseline for follow-up to the susceptibility trend of various drugs to be used for the treatment of *S. aureus* infections. Routine screening of MRSA and regular studies should be conducted to find out the sources of MRSA. Culture and susceptibility testing is when *S. aureus* is suspected as etiological bacterial agent which greatly improves quality of patient management. We recommend further research on molecular studies to evaluate the resistance genes and monitoring the epidemiology of multiple drug resistant *S. aureus* and MRSA. In addition, further studies on healthcare workers is highly commended to assess the potential role in MRSA transmission.

We further indorse the use of antibiotics only to serious bacterial infections and prescribed by physicians basing on laboratory advise and patients completing a full course of antibiotics as instructed by physician in order to help forestall the spread of antibiotic resistant bacteria.

There is urgent need to reduce or eliminate the preventative use of antibiotics on livestock and crops in order to curb the further evolution of resistant strains. More research is recommended towards development of new antibiotics in order to win the battle against multidrug resistant bacterial strains like MRSA.

PUBLICATIONS

Journal Articles

1. **Andrew, B.**, Tibyange, J., Owalla, T., Atwiine Kagirita, A., & Bazira, J. (2019). *Highly Resistant Staphylococcus aureus Isolated from Patients Attending a Tertiary Hospital, South Western Uganda. Microbiology Research Journal International* (Vol. 26). https://doi.org/10.9734/MRJI/2018/44939
2. **Baguma Andrew,** Atek Atwiine Kagirita, Owalla Tonny, Joel Bazira (2018). *High Rates of MRSA Colonization of Domesticated Swine of Kabale District – South-western Uganda, Journal of Advances in Microbiology* 10(3):1-9, 2018; Article no. JAMB.41085ISSN:2456-7116
3. **Baguma Andrew**, Atek Kagirita, and Joel Bazira, "*Prevalence of Extended-Spectrum Beta-Lactamases-Producing Microorganisms in Patients Admitted at KRRH, South-western Uganda,*" *International Journal of Microbiology*, vol. 2017, Article ID 3183076, 5 pages, 2017. doi:10.1155/2017/3183076
4. **Baguma Andrew**, Kiiza Aidah and Joel Bazira (2016). *Prevalence of Methicillin Resistant* Staphylococcus aureus *among Isolates from Wounds in Surgical Wards at Kabale Regional Referral Hospital, South Western Uganda. British Microbiology Research Journal*, ISSN: 2231-0886, Vol.: 17, Issue.: 5

Manuscripts in development

2. ***Baguma Andrew****, *Orikiriza Patrick[1], Atek Kagirita, Owalla Tonny and Bazira Joel* Phylogenetic relationship between Methicillin Resistance Staphylococcus aureus from humans and animals in Kabale District - South western Uganda.

Conference Papers

1. ***Baguma Andrew,*** *Benon Asiimwe, Bazira Joel (2017).* Efficacy of Cefoxitin disc diffusion test as surrogate marker for Methicillin resistance in comparison to mecA gene PCR to detect MRSA.

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APPENDICES

Appendix 1. Gel electrophoresis of mecA gene amplified by PCR



The result of PCR and gel electrophoresis for mecA gene Amplicon of approximately 180bp as expected from MRSA and MSSA with Positive control (MRSA ATCC 43300) and negative control (MSSA ATCC 25923) and selected *S. aureus* isolated during the study. L is 100bp ladder,1- Positive control (MRSA ATCC 43300), 2 – negative control (MSSA contains a Methicillin Susceptible S. aureus strain ATCC 25923) Lanes 3, 4,5,6,7,9,11,12,1314 and15 mecA positive (MRSA) from clinical samples of inpatients (3,4 and5), OP (6 and 7), HHC (9 and 10), Cattle (11 and 12) and swine (14 and 15). Lane 8, 10 and 16 mecA negative (MSSA) isolates from clinical samples. L2 is the Negative amplification control.

Appendix 2. Blood Agar Plate Medium Preparation QC Form

QC Strains

QC Organism	ATCC™	Lot No.	Manufacture Exp. Date	Stock Culture Date
Staphylococcus aureus	25923			
Escherichia coli	25922			

QC Result Sheet

Date/Initial	Batch#	pH	Sterility Check	QC Organism-Result		
				S. aureus (25923)	*E. coli* (25922)	
Initiated: Read:			□ Pass □ Fail	□ Growth □ No Growth Hemolysis: □ beta □ alpha □ gamma	□ Growth □ No Growth Hemolysis: □ beta □ alpha □ gamma	□ Pass □ Fail

Comments: _____ **Performed by (Initials/Date):** _____

Reviewed by (Initials/Date): _____

Appendix 2. Gram Staining Quality Control Form

Prepared Date: _____ **Expiration Date:** _____

Reagent	Lot Number	Expiration Date	Received Date
CRYSTAL VIOLET			
LUGOLS'IODINE			
ACETONE			
NEUTRAL RED			

QC Organism	ATCC™	Expected Result	Pass/Fail
Staphylococcus aureus	25923	**Dark purple organisms**	
Escherichia coli	25922	**Pale to dark red organisms**	

Comments:

Appendix 3. Quality Control Sheet for in house prepared Mueller Hinton agar (MHA)

Prepared Date: _____ Expiration Date: _____Prepared by (Initial/Date): ___

Reagent	Cat Number	Lot Number	Expiration Date	Quantity Added
MHA				

pH Adjustment Reagents **N/A** ☐

Reagent	Catalog Number	Lot Number	Expiration Date
1N NaOH			
1N HCl			

QC Organism	ATCC™	Lot No.	Manufacture Exp. Date	Stock Culture Date
Staphylococcus aureus	25923			
Escherichia coli	25922			
Pseudomonas aeruginosa	27853			

STERILITY QC

Date/Initial	Batch#	pH	Sterility Check	QC Organism-Result			QC Pass/Fail
				S. aureus (25923)	*E. coli* (25922)		
Initiated: Read:			☐ Pass ☐ Fail	☐ Growth ☐ No Growth	☐ Growth ☐ No Growth	☐ Growth ☐ No Growth	☐ Pass ☐ Fail

Appendix 4: Disc diffusion: QC ranges (clsi) for staphylococcus aureus atcc 25923

Antibiotic	Disk Potency (µg)	Zone ranges (mm)	Size of zone (nearest whole mm)	Result: Pass or Fail
Amikacin	30	20-26		□ Pass □ Fail
Ampicillin	10	27-35		□ Pass □ Fail
Azithromycin	15	21-26		□ Pass □ Fail
Cefoxitin (surrogate test for Oxacillin)	30	23-29		□ Pass □ Fail
Chloramphenicol	30	19-26		□ Pass □ Fail
Ciprofloxacin	5	22-30		□ Pass □ Fail
Clindamycin	2	24-30		□ Pass □ Fail
Doxycycline	30	23-29		□ Pass □ Fail
Gentamicin	10	19-27		□ Pass □ Fail
Nitrofurantoin	300	18-22		□ Pass □ Fail
Tetracycline	30	23-24		□ Pass □ Fail
Trimethoprim-Sulfamethoxazole	1.25/23.75	24-32		□ Pass □ Fail

Appendix 5. Research Approval letter from Mbarara University of Science and Technology Research Ethics Committee(MUST –REC)

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
P.O. Box 1410, Mbarara Uganda. Tel: +256 485433795; Fax: +256 4854 20782
RESEARCH ETHICS COMMITTEE
E-mail: sec.rec@must.ac.ug

Ref: MUREC 1/7

Date: October 30, 2017

Mr. Andrew Baguma
Post graduate student

Re: **Request for renewal of approved study on "Origins and spread of methicillin-resistant *staphylococcus aureaus* in Kabale District-South western Uganda" N0. 13/08-15**

Type: [] Initial Application
[ ] Protocol Amendment
[ ] Letter of Amendment (LOA)
[√] Continuing Review
[ ] Material Transfer Agreement
[ ] Other, specify: _____

Your progress report and request for renewal for the above mentioned study has been received and reviewed by MUST REC.

I write to inform you that renewal has been granted for a period of one year up to October 30, 2018.

Sincerely,

Dr. Francis Bajunirwe
CHAIR,
MUST RESEARCH ETHICS COMMITTEE

RESEARCH ETHICS COMMITTEE
MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
P.O. BOX 1410, MBARARA

Appendix 6. Research Approval letter from National Council of Science and Technology (UNCST)

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 2172 **29th April 2019**

Mr. Andrew Baguma
Principal Investigator
C/o Mbarara University of Science and Technology
Mbarara

Dear Mr. Baguma,

Re: Research Approval: Origins and Spread of Methicillin – Resistant Staphylococcus Aureus In Kabale District – South Western Uganda

I am pleased to inform you that on **12/12/2017**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **12/12/2017** to **12/12/2018.**

Your research registration number with the UNCST is **HS 2172.** Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

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Uganda National Council for Science and Technology
(Established by Act of Parliament of the Republic of Uganda)

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	N/A	January 2015
2.	Informed consent documents	English and Runyankore	N/A	N/A
3.	Informed assent documents	English	N/A	N/A

Yours sincerely,

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Chair, Mbarara University of Science and Technology, Research Ethics Committee

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Appendix 7. Letter of introduction to respondents

Department of Microbiology,

Faculty of Medicine,

Mbarara University of Science and Technology,

P. O. Box 1410,

Mbarara.

22nd April, 2016

Dear Prof./Assoc. Prof./Dr./Mr./Mrs./Ms.....

RE: ORIGIN AND SPREAD OF METHICILIN RESISTANCE STAPHYLOCOCCUS AUREUS IN GREATER KABALE

Staphylococcus aureus, is a bacterial germ common in nature and human flora and can cause diseases in humans and animals. It can spread from human to human or to animals and vice versa causing various clinical situations varying between superficial infections and serious life-threatening infections like severe sepsis, pneumonia, and toxic shock syndrome (TSS). Another potential feature of this bacteria especially MRSA, is its potential to develop antimicrobial resistance to various antibiotics. This results into serious infections that are hard to treat among humans (both community and hospitals) and your animals.

As part of a Ph. D study, I am carrying out a survey to find out its origin and how it spreads among humans and animals (especially domestic Cattle and Swine) in greater Kabale. You have been identified as one of the respondents for the survey because you own the mentioned domestic animals. I am, therefore, requesting you to allow me take nasopharyngeal swabs from you, other family members you stay with and your domestic cattle, Swine or both. There is no incentive attached to this process and your acceptance is voluntary with no violation of your rights. The success of this study depends largely on your acceptance to take specimens as per the study protocol by the study assistants. The laboratory results of you and your animals will be will be treated with ***STRICT CONFIDENTIALITY***. It is hoped that the results of this study will serve as a guide by policy makers and health care professional to

develop improved prevention and control measures to reduce or stop the spread within the community and hospital settings.

I thank you very much for your positive responses.

Yours faithfully,

Baguma ANDREW (AB)

Ph. D. Candidate, Registration No. 2014/PHD/004.

Contact phone: +256-752202068//0783810600

Appendix 8. HRM REPORT



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Melt Report

2. Experiment Information

Run Name	Jupiter_Baguma_Spa HRM_Run one
Run Start	8/6/2018 5:06:32 PM
Run Finish	8/6/2018 8:09:36 PM
Operator	jupiter/Baguma
Notes	
Run On Software Version	Rotor-Gene Q Software 2.3.1.49
Run Signature	The Run Signature is valid.
Gain Green	5.
Gain HRM	2.
Machine Serial No.	100886

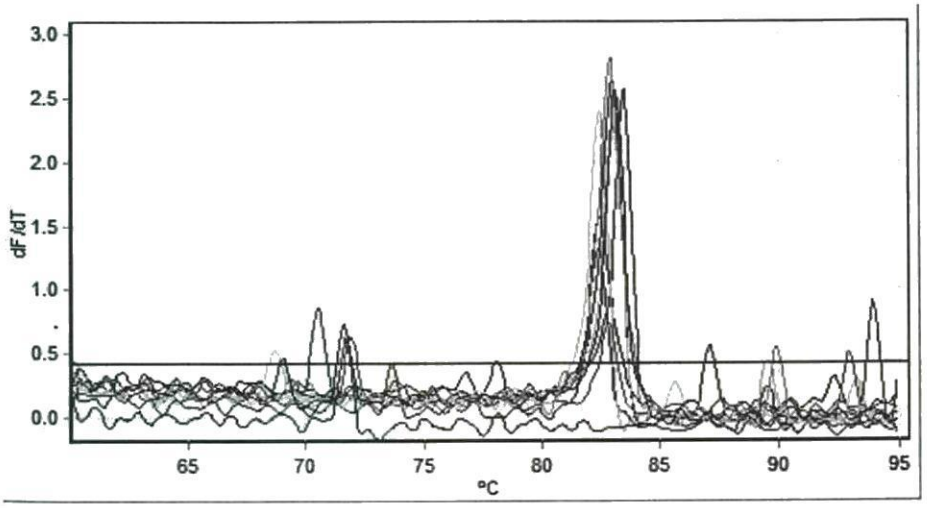
3. Melt Information

Digital Filter	Light
Imported Analysis Settings	
Sample Page	Page 1
Temp. Threshold	0°C
Threshold	0.42106

4.Profile

Cycle	Cycle Point
Hold	Hold @ 95°C, 10min 0s
Cycling (45 repeats)	Step 1: Hold @ 95°C, 10s
	Step 2: Hold @ 60°C, 15s
	Step 3: Hold @ 72°C, 25s, acquiring to Cycling A([Green][1][1])
	Step 4: Hold @ 40°C, 60s
Melt	Ramp from 60°C to 95°C
	Hold for 90s on the 1st step
	Hold for 1s on next steps,Melt A([HRM][7][1])

5.Melt data for HRM A.HRM



No.	Color	Name	Genotype	Peak 1	Peak 2	Peak 3	Peak 4
3		OP 158		83.00			
4		OP 33		73.70	82.40		
11		KMCCHH 165		78.10	82.44		
22		KMCC HH/1H		82.94	89.86		
27		OP 123		70.54	83.50	87.10	
29		3457/17		68.70	82.44		
32		KMCCHH 14H		71.94	82.84	92.90	93.90
34		2722/17		83.06	89.50		
35		+VE CTRL		69.04	71.64	83.14	
36		-VE CTRL		71.70			

Bin Name	Temperature	Sample No.	Sample Name	Peak

This report was generated by Rotor-Gene Q Series Software 2.3.1 (Build 49)
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Appendix 9. Map of greater Kabale region where the study was conducted





British Microbiology Research Journal
17(5): 1-5, 2016; Article no.BMRJ.27807
ISSN: 2231-0886, NLM ID: 101608140

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Prevalence of Methicillin Resistant *Staphylococcus aureus* among Isolates from Wounds in Surgical Wards at Kabale Regional Referral Hospital, South Western Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Author BA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors BA, KA and JB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/27807
Editor(s):
(1) Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.
(2) En Tao Wang, Departamento de Microbiologia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México and State Key Laboratory of Agrobiotechnology and College of Biological, Sciences, China Agricultural University, Beijing, China (Invited Professor).
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(4) Isabelle Luz, Oswaldo Cruz Foundation, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/16945

Original Research Article

Received 20th June 2016
Accepted 28th October 2016
Published 17th November 2016

ABSTRACT

Background: Methicillin resistant *S. aureus* (MRSA) is a multidrug resistant organism that threatens the continued effectiveness of antibiotics worldwide and causes a threat almost exclusively in hospitals and long-term care settings. This study sought to determine the prevalence and antimicrobial susceptibility pattern of MRSA at Kabale Regional Referral Hospital (KRRH) in

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South Western Uganda.
Methods: This was a cross sectional study conducted between June and November 2014 involving 350 pus swabs from infected surgical sites. The samples were cultured on Mannitol salt agar at 37℃ for 17- 24 hours and the colonies were tested and confirmed as *S. aureus* using API staph testing kit. Screening for MRSA was performed using the Cefoxitin (30 µg) disc on Muller Hinton agar medium. MRSA susceptibility was performed by the Kirby Bauer disc diffusion on MHA for the following antibiotics: Ceftriaxone, Ciprofloxacin, Erythromycin, Imipenem, Trimethoprim – sulfamethoxazole, Levofloxacin and Gentamycin. The zones of inhibition were measured using a ruler and compared with the CLSI guidelines 2012.
Results: From the 350 pus swabs, 331 isolates of *S. aureus* were obtained, of which 179 (54%) isolates were Methicillin resistant. Majority (54%) of the MRSA isolates were highly sensitive to Gentamycin and Levofloxacin but highly resistant to Ceftriaxone (100%), Imipenem (100%), Trimethoprim – sulfamethoxazole (85%), and Erythromycin (67%).
Conclusion: The prevalence of MRSA in KRRH is high with high resistance to Ceftriaxone, Imipenem, Trimethoprim – sulfamethoxazole and Erythromycin. However, good sensitivity to gentamycin and Levofloxacin is still observed.

Keywords: Methicillin resistant Staphylococcus aureus (MRSA); Kabale Regional Referral Hospital (KRRH); surgical ward.

1. BACKGROUND

S. aureus is a versatile pathogen capable of rapidly acquiring antibiotic resistance and a notable characteristic of this bacterium is the Methicillin resistant *S. aureus* (MRSA) which has adapted to survive treatment with beta lactam based antibiotics including Methicillin or Nafcillin. MRSA is now responsible for several difficult-to-treat infections in humans [1]. Infections with MRSA are of special concern because these infections are associated with prolonged hospital stay, increased hospital costs, and have a few therapeutic options for affected patients [2].

Although MRSA is reportedly becoming a predominant cause of nosocomial infection in the Ugandan hospital settings resulting in considerable morbidity and mortality, its prevalence in rural settings has not been properly studied. Therefore, this study sought to determine the prevalence of MRSA among the *S. aureus* isolates and its antimicrobial susceptibility pattern on the commonly used antibiotics at KRRH, which is located in rural South Western Uganda, bordering Uganda and Rwanda and not very far from the Democratic Republic of Congo.

2. MATERIALS AND METHODS

This was a cross sectional study conducted in a period of six months, between June and November 2014 involving 350 pus swabs from female and male patients of all ages with septic surgical site and admitted at the surgical ward of KRRH. Sterile dry cotton swabs were used to collect pus from patients who consented. The swabs were rolled gently but firmly on the base of the surgical wound while applying an even pressure. The pus swabs were then transported to the KRRH laboratory immediately where they were directly inoculated onto Mannitol Salt Agar (Mast Group Ltd) and incubated at 35 - 37℃ for 18 – 72 hours. *S. aureus* bacteria were identified by Gram staining, Catalase, Coagulase reactions and confirmed with API staph testing kit [3]. The isolated *S. aureus* were subjected to cefoxitin (30 µg) antibiotic disc to determine the Methicillin resistance by the disc diffusion method on Mueller–Hinton agar plates, using *S. aureus* bacterial suspension with the turbidity adjusted to a 0.5 McFarland standard. Plates were also incubated at 35 – 37℃ for 24 hours. Results were interpreted according to clinical and laboratory standard institute (CLSI) guidelines where interpretive criteria for cefoxitin sensitive or resistant *S. aureus* was ≥ 25 mm and ≤ 24 mm zones of diameter respectively. We used the modified Kirby-Bauer technique, [4,5], to determine susceptibilities of MRSA isolates on to the commonly used antibiotics at KRRH. The antibiotics included Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Erythromycin (15 µg), Cotrimaxazole (1.25/23.75 µg) and Levofloxacin (5 µg). The zones of inhibition were read and measured using a meter ruler in mm [4] and later recorded into the data collection form. The generated data was analyzed using SPSS 21.0 Armonk: NY BM Corp. to give descriptive statistics which included frequencies, percentiles and ratios.

2.1 Quality Control

For quality control, we used standard *S. aureus* reference strains, including the methicillin sensitive strains *S. aureus* (MSSA) ATCC 25923 and ATCC 29213, and a methicillin resistant strain *S. aureus* (MRSA) ATCC 43300. These controls were procured from the Uganda National Health Laboratories (UNHLs) formerly known as Central public health laboratories (CPHL).

2.2 Ethical Considerations

Ethical approval was obtained from the Institutional Research ethics committee (IREC) of Kampala international University (KIU) as well as ethics and research committee of KRRH. Informed consent was also sought and obtained from the patients. All results were treated with utmost confidentiality.

3. RESULTS

In this study, 350 pus swabs were collected from patients admitted on surgical wards of KRRH. Three hundred and thirty one (94.6%) isolates were *S. aureus*, among which 179 (54%) were phenotypically resistant to methicillin (MRSA). All MRSA were later tested for susceptibility to seven commonly used antibiotics (Ceftriaxone, Imipenem, Erythromycin, Ciprofloxacin, Cotrimaxazole, Levofloxacin and Gentamycin). MRSA isolates showed high range of cross-resistance to the seven tested antibiotics (Fig. 1). As illustrated in Fig. 1, ninety six [96 (54%)] of the MRSA isolates were most susceptible to Levofloxacin and least susceptible to Ceftriaxone, Imipenem and Erythromycin. All the 179 MRSA isolates were resistant to Ceftriaxone and Imipenem; while 152 (85%), 120 (67%) and 117 (65%) were resistant to Trimethoprim – sulfamethoxazole, Erythromycin, and Ciprofloxacin, respectively. It was observed that most MRSA isolates were susceptible to Levofloxacin (96 isolates, 54%) and Gentamycin (142 isolates, 80%).

4. DISCUSSION

S. aureus is one of the common bacterial pathogen causing infection in the hospital settings and the individuals at risk are especially those with surgical wounds [6]. In a duration of 4 months, 350 pus swab specimen were cultured and one hundred and seventy nine (54%) of the bacterial isolates were *S. aureus*, indicating the colonization nature of this bacterium to the hospitalized patients and that the patients with surgical wounds are at an increased risk [7]. *S. aureus* is a common pathogen that has hindered antibiotic therapy and MRSA is the most common pathogen in surgical site infections causing extended hospital stays and increased health-care costs [6]. This nature of colonization has been reported previously [7]

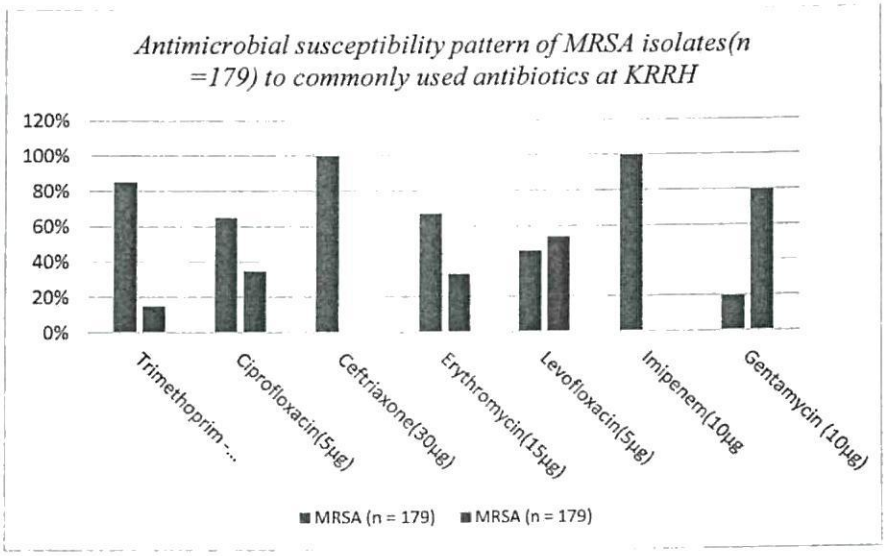


Fig. 1. Antimicrobial Susceptibility pattern of *S. aureus* isolates (n = 179)
n = total number of isolates; MRSA = Methicillin resistant S. aureus

In this study we observed a high ratio of MRSA among the isolated *S. aureus*, similar to what was reported previously in Ugandan hospitals like Mulago National hospital [4] and Mbarara Regional referral Hospital [5]. To control MRSA, patients should be treated with appropriate antibiotics and strict implementation of infection control measures. These actions are currently not being implemented in our hospital settings probably due to attitude of prescribers like clinicians and the nursing staff.

MRSA isolates were less susceptible to commonly used antibiotics like Ceftriaxone, Imipenem, Ciprofloxacin, Erythromycin and Trimethoprim – sulfamethoxazole. However, most of them were still susceptible to the antibiotics Gentamycin and Levofloxacin, but this susceptibility is steadily decreasing at KRRH which is alarming since the two antibiotics are always used to treat MRSA [8]. Several factors could be associated with this high degree of resistance, which is pertinent with other rural hospitals in Uganda. These factors may include lack of access to clean water for frequent bathing, poly pharmacy, and ward overcrowding [9]. These scenarios are of serious public health concern and continue to signify a therapeutic challenge to clinicians while treating patients. However, improved hygiene through frequent patient bathing and hand washing by health care workers can greatly reduce MRSA spread [10]. In situations where diagnostic tools are limited, clinicians are left with no choice but to irrationally prescribe many antibiotics to patients for quicker prognosis. However, resistance to antibiotics is a major public-health concern and irrational use antibiotic is the major driving force to resistance [11]. Therefore, the antibiotic prescription tradition with correct diagnosis and the usage of antibiotics requires to be critically considered. Overcrowding is a relevant factor in MRSA spread within hospitals [12], which lead to failure of MRSA control programs. This can result into high MRSA incidences leading to increased inpatient length of stay exacerbating, the situation leading further infection control failure.

5. CONCLUSIONS

S. aureus is a common clinical bacterium isolated from patients at surgical ward at KRRH, with a high prevalence of MRSA. Most of the MRSA isolates are resistant to Ceftriaxone, Imipenem, Trimethoprim – sulfamethoxazole and Erythromycin, but present good sensitivity to gentamycin and Levofloxacin.

6. RECOMMENDATION

We recommend proper antibiotic prescription policies based in local resistance data and enforced by the appropriate authorities to reduce the abuse of antibiotics and reduce the acquisition of resistance by pathogens. More to that, a stronger antibiotics effective to MRSA should be included on the treatment menu and be procured for availability to prescribing health care workers. An effective infection control team should be formed and be given mandate to function.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16945



Journal of Advances in Microbiology

10(3): 1-9, 2018; Article no.JAMB.41085
ISSN: 2456-7116

High Rates of Methicillin-Resistant *Staphylococcus aureus* Colonization of Domesticated Swine of Kabale District – Southwestern Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Author BA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AK and OT managed the analyses of the study. Author BJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/41085
Editor(s):
(1) S. Pradeep, Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Kerala.
Reviewers:
(1) Syed Umer Jan, University of Balochistan, Pakistan.
(2) İlknur Dağ, Eskisehir Osmangazi University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/24547

Original Research Article

Received 15th February 2018
Accepted 24th April 2018
Published 9th May 2018

ABSTRACT

Background: *S. aureus* is a commensal mammalian pathogen which can establish itself as part of the skin flora. However, can eventually cause infections and invasive diseases in both hospital and community settings. Livestock-associated Methicillin-resistant *S. aureus* remains a major concern to public health. This study investigated the rates of methicillin resistance *S. aureus* (MRSA) colonization and respective antibiotic resistance profiles in domestic pigs in Kabale District - South Western Uganda.

Method: This was a cross-sectional study conducted between June 2016 and February 2017 in which nasal swabs from 585 pigs from 147 homesteads were collected and cultured using standard

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microbial techniques to isolate *S. aureus* and phenotypically screen for MRSA using Cefoxitin disc. MecA polymerase chain reaction (PCR) was used to confirm MRSA. Antimicrobial susceptibility testing was performed using the Kirby Bauer technique to determine antimicrobial susceptibility pattern among MRSA towards to the commonly used antibiotics in the region.

Result: From the five hundred and eighty-five (585) pigs, 172 (29.4%) were MRSA. There was high antibiotic resistance among MRSA isolates was observed against Sulfamethoxazole – trimethoprim was 170(99%), Erythromycin; 154(89%), Ciprofloxacin 124(72%), Clindamycin; 121(70), Tetracycline; 121(70%), Gentamycin; 84(49%), Rifampicin; 40(23%); Cefipime; 40(23%) and Vancomycin; 03(2%).

Conclusion: The observed high rate of MRSA colonization among domestic pigs is of a significant public health concern in Kabale region. A greater number of MRSA isolates were highly resistant to commonly used antibiotics.

Keywords: MRSA; Swine; Uganda.

1. INTRODUCTION

S. aureus is a commensal mammalian pathogen, which establishes itself to the skin as a flora, and eventually causes infections and invasive diseases to both hospital and community settings [1,2]. *S. aureus* is never an absolute commensal since it turns to be pathogenic to man and many homoeothermic species [3]. The invasiveness of this species is enhanced by its capacity to produce a complex arsenal of toxins and ability to acquire resistance to multiple antimicrobials [4,5]. *Staphylococcus aureus* acquisition of mobile genetic element, staphylococcal cassette chromosome (SCC*mec*) carrying *mecA* gene which encodes for an altered PBP – PBP2a/PBP2' which results into a high level of resistance to β-lactam antibiotics in MRSA [6]. The expressed protein, which is a transpeptidase, catalyzes cell-wall crosslinking in the face of the challenge by β-lactam antibiotics. This results into a reduced affinity for β-lactam antibiotics and therefore, cell wall biosynthesis in MRSA strains continues even in the presence of otherwise inhibitory levels of β-lactam antibiotics. The activity of this protein is regulated by allostery at a site 60 Å distant from the active site, where crosslinking of cell wall takes place [7]. Methicillin-resistant *S. aureus* (MRSA) is of increasing importance in hospitals [8] the community [9] and livestock farming [10]. To date, livestock-associated MRSA (LA-MRSA) has been found worldwide, particularly among people who are involved with livestock farming [11]. Of particular concern, is the MRSA which causes difficult-to-treat infections leading to increased length hospitalization and mortality [12,13]. The widespread emergence of MRSA has continued to be a serious global public health concern especially in hospitals [14] communities [15] and in animals as livestock-associated Methicillin-resistant *S. aureus* (LA – MRSA) [16]. Animal-to-human transmission of MRSA have been reported and the high prevalence of MRSA puts the community at high risk to invasive and hard to treat infections [17]. Although there is an increasing level of swine domestication as a source income in Uganda [18,19]. There is no data on colonization of these animals with S. aureus including MRSA. This study was conducted to determine the colonization rates of *S. aureus* including MRSA and to determine the resistance patterns of the bacterial strains to the common antibiotics used to treat human infections.

2. MATERIALS AND METHODS

2.1 Sample Collection

This was a cross-sectional study conducted between June 2016 and February 2017 and involved147 homesteads in Kabale district with domestic pigs. After farmers consented, nasal swabs from 585 pigs were collected using sterile cotton-tipped swabs and transported in Amie's transport medium (Hi-Media, Mumbai) for bacteriological analysis in Microbiology laboratory located at Uganda national health laboratories (UNHLS), formerly Central public health laboratories (CPHL).

2.2 Bacteriological Analysis

This involved immediate inoculation of the swabs into 7.5% NaCl (w/v) in Nutrient broth (NB) (Difco, Detroit, MI) with a slight modification of Chatterjee et al. methodology (20) and incubated overnight for enrichment to *S. aureus*. A discrete colony growth on the NB was sub cultured onto mannitol salt agar (Difco, Detroit, MI) and further incubated aerobically at 37°C for 24 hours.

S.aureus growth was identified as red colonies [21], which were further characterized by catalase, tube coagulase [22,23] and DNase test [24,25] (Hi-Media, Mumbai). API STAPH (bioMerieux, Inc., Durham, NC) was used following manufacturer's instructions [26] to phenotypically confirm *S. aureus*. The isolates were screened for methicillin resistance using Cefoxitin 30 ug disks diffusion techniques as described in the CLSI, 2015 guidelines [27]. To achieve this, a suspension of *S. aureus* isolates equivalent to 0.5 McFarland was inoculated onto Mueller-Hinton agar followed by application of Cefoxitin (30 µg) disk and the inoculated plates were incubated aerobically at 35°C for 18-24 hours. The zone of inhibition diameter around Cefoxitin (30 µg) disk was measured using a Vernier calliper and results recorded and compared with the CLSI, 2015 standards. Any *S. aureus* isolates with zone diameter of ≤ 21 mm was considered to be MRSA while similar isolate with ≥ 22 mm was considered as methicillin sensitive *S.aureus* (MSSA) [27].

2.3 Molecular Testing

Phenotypic MRSA isolates were confirmed by PCR assay to detect mecA gene [28]. DNA was extracted from all the phenotypic MRSA previously sub-cultured on brain heart infusion (Difco Laboratories) agar plates as purity plates and incubated at 37°C, after 24hours. One colony was suspended in 25 µl of sterile distilled water and the suspension was then placed in a 100°C heat block for 15 min according to Pérez-Roth et al. [29]. A 5-µl volume was directly used as a template for PCR amplification. For MecA, the following primers were used "MecA F; 5' TCC AAT TAC AAC TTC ACC AGG 3' and "MecA R; 5' CCACTTCATATCTTGTAA CG 3'" as described by Simone *et al* (30). PCR products were detected by gel (electrophoresis followed by visualization using UV transilluminator (Fig. 1).

2.4 Antimicrobial Susceptibility Testing

Isolates were tested for their antimicrobial susceptibility by disk diffusion test (31). The drugs tested were those commonly used in the Kabale region and recommended in the Uganda clinical guideline(UGC)(32), including Ciprofloxacin (CIP), chloramphenicol (C), clindamycin (CL), Erythromycin (E), gentamicin (CN), Rifampin (RIF), tetracycline (TE), Sulfamethoxazole - trimethoprim (SXT), Ceftaroline and vancomycin (V). A 0.5 McFarland standard suspension of all isolates was prepared and a lawn culture of bacteria seeded on to Mueller-Hinton agar plates (Difco, Detroit, MI) where a panel of antibiotic disks was placed according to Kirby Bauer disk diffusion technique

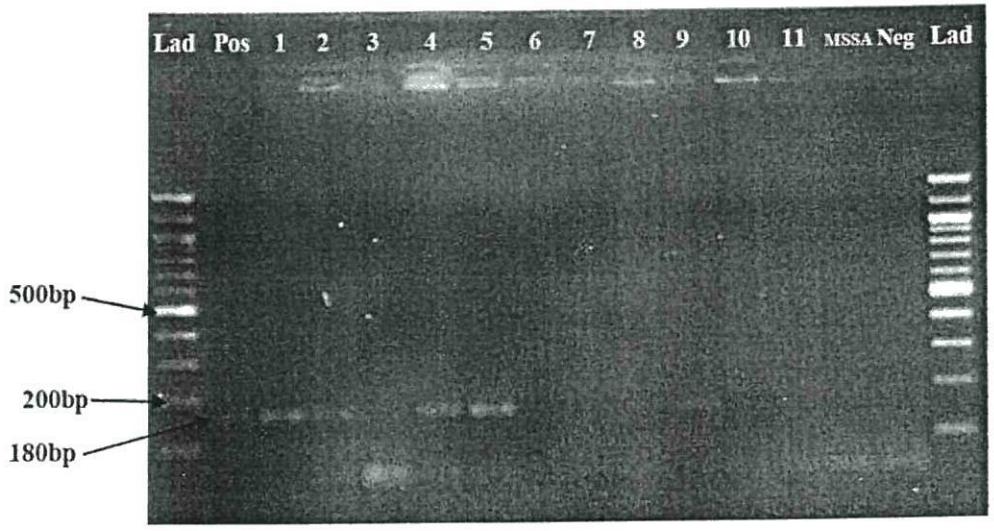


Fig. 1. Results of mecA gene PCR where an Amplicon of approximately 180bp was expected. The lad is a 100bp ladder, Lane Pos. contains the Positive control (MRSA ATCC 43300), and Lanes 1, 2,3,4,5, and 9 contain MRSA isolates from swine. Lane 6, 7,8,10 and 11 were MSSA isolates from swine. MSSA contains a Methicillin-Susceptible S. aureus strain ATCC 25923, whereas Neg. is the Negative amplification control

following recommendations in the Clinical Laboratory Standard Institute (CLSI 2015)(33) at 37°C [27]. In order to be able to detect inducible clindamycin resistance (iCR), Clindamycin (2 ug) and Erythromycin (15ug) antimicrobial disks (Oxoid™) were positioned at a distance of 15mm (edge to edge) from each other during every run of Kirby Bauer disk diffusion test to determine induced clindamycin resistance among isolates. Resistance pattern was confirmed with VITEK® 2 (BioMerieux France) an automated instrument for identification and antibiotic sensitivity testing [34,35]. Every isolate that was vancomycin resistance by disk diffusion technique, was further suspended to 1.0 McFarland Turbidity Standard (Fisher Scientific, R20411) in 0.85% NaCl – saline (BioMerieux France) and tested using FilmArray Blood Culture Identification Panel following manufacture instruction to confirm the presence of vanA/B -vancomycin-resistance genes [36,37].

2.5 Quality Control

Only in-house prepared media was used in this study, *and S. aureus* (ATCC™ 6538), *S. epidermidis* (ATCC 12228), *Proteus mirabilis* (ATCC 12453), and *Escherichia coli* (ATCC 25922) were used following manufacturer's instructions for every new batch of MSA [38] prepared before use. MSSA (ATCC 25923) and *MRSA*) ATCC 43300 were used as negative and positive controls respectively for every new batch of Mueller-Hinton agar plates (Difco, Detroit, MI) prepared before antibiotic susceptibility testing. All antibiotic used were procured from Oxoid and underwent quality control according to the manufactures instructions.

2.6 Ethics Statement

The Institutional Review committee of Mbarara University of Science and Technology (MUST) and the Uganda National Council of science and technology (UNCST) approved the protocols (Number 13/08 – 15) prior to the initiation of the study. All the farmers consented before the samples were collected from their animals.

3. RESULTS

Of the five hundred and eighty-five (585) pigs that were sampled, 254 (43.4%) were found to be colonized by S. *aureus*. MRSA was confirmed in 172(29.4%) by cefoxitin disk diffusion test and *mecA* gene detection by PCR. As shown in Fig. 2, the MRSA isolates were phenotypically highly resistant to erythromycin, Cotrimoxazole, ciprofloxacin, clindamycin and tetracycline. Of note, three isolates (1.9%) were resistant to vancomycin. Antibiotic resistance was high among MRSA compared to MSSA isolates (Fig. 2) where the resistance against Sulfamethoxazole – trimethoprim was 170(99%), followed by Erythromycin; 154(89%), Ciprofloxacin 124(72%) and Clindamycin; 121(70), Tetracycline; 121(70%), Gentamycin; 84(49%), Rifampicin; 40(23%); and Cefipime; 40(23%). Of note, 03(2%) of the MRSA isolates were resistant to Vancomycin. However, among the MSSA, there was low antibiotic resistance compared to MRSA (Fig. 2).

4. DISCUSSION

The present study provides novel information about *S. aureus* including MRSA *carriage* rate among pigs in Kabale district- South Western Uganda. Currently, Pig farming is an important activity that provides local population with an opportunity to generate house hold income [39]. Increasing demand for pork has resulted in fast-growing market for this animal, consequently, many families are acquiring these animals as an income generating venture. However, this might come with public health cost because of the potential to act as a reservoir for drug-resistant human pathogens. The zoonotic spread of MRSA to humans through direct animal contact, environmental contaminations are of great concern [40,41] where such phenomenon could undermine existing MRSA control programs. The close proximity nature of farmers and domestic pigs in their respective homesteads provides high chances of close contact which may result into cross-transmission [6,15,20,42,49]. However, until recent focus has been researching on *S. aureus* in swine neglecting MRSA and its ancestral origin [43] as this may be too costly in resource-limited settings. Consequently, this study, only did a limited molecular analysis to establish *mecA* gene but not phylogenetics and therefore difficult to establish the origin of the colonizing type which would have given insight as to whether it was livestock-associated MRSA or the human type and likely route of transmission.

MRSA colonization has recently been identified in pigs and people that work with pigs, raising concerns about the role of pigs as reservoirs of MRSA for human infection [44,17]. Mounting concerns regarding the occupational and public health implications of MRSA in pigs and other

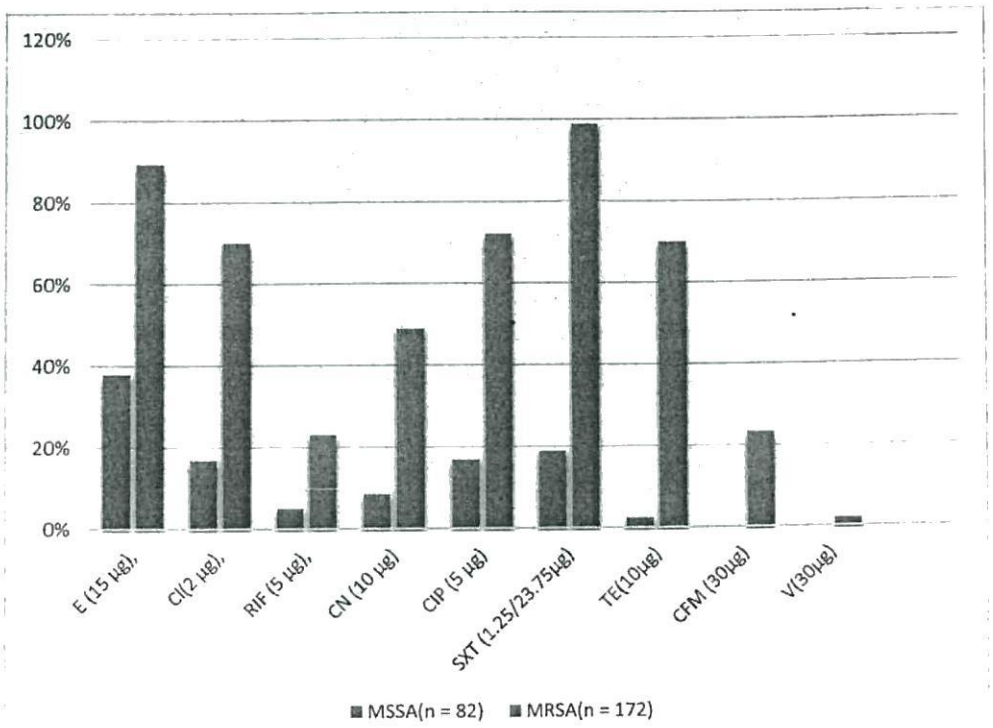


Fig. 2. The resistant pattern of *S. aureus* isolates

Note: n= number; MSSA = Methicillin-susceptible S. aureus; MRSA = Methicillin resistance S. aureus, S = Sensitive, R =Resistant, E = Erythromycin, CL= Clindamycin, RIF = Rifampicin, CN = Gentamycin, CIP = Ciprofloxacin, SXT = Sulfamethoxazole – trimethoprim, TE = Tetracycline, CFM =Cefipime, V = Vancomycin

livestock populations requires intensive research of MRSA in animals, and particularly pigs which are increasingly reared as a source of income in Uganda. For instance, the colonization rate of 29.4% is alarmingly high, and this is consistent with other studies which demonstrated similar colonization rates of this pathogen in swine population [45,46]. In addition, this also similar to Mroczkowska et al. [47] and Van Cleef et al. [48] who reported carriage of MRSA to be 29% and 30% in Demark and Thailand respectively. The reasons for this high prevalence are unknown, but might be related to the altered interplay of the host, the microorganism, and the environment. In addition, this may demonstrate the abuse of the antibiotics that are supposed to be used in human but are now freely and generously used in animals [49,50]. The use of antibiotics in livestock farming is routinely described as probably the major contributor to the antimicrobial resistance phenomenon being witnessed today in human medicine [50]. The MRSA prevalence reported in this study is much higher than that previously reported in Dakar – Senegal, United states (USA) [51] and the Netherlands [52]. However, the prevalence of MRSA in pig herds varies widely (0 to 50%) among European countries [52,53]. The pig herd prevalence of MRSA in North America is uncertain, but appears lower than in many European countries [54]. The prevalence and spread of MRSA in Africa is unclear, in contrast to the rest of the world [55]. However, by Kalule et al. (2014) reported MRSA prevalence rate of 64% among *S.aureus* isolated from pigs in Uganda [56].

Alarmingly, studies about the epidemiology of S. aureus and MRSA associated with swine has been remarkably neglected and considerable uncertainty remains regarding MRSA prevalence among pigs in Uganda. The high prevalence of MRSA in the pigs demonstrates a potential public health threat with great risks of zoonotic transmission of the MRSA to humans and vice versa. MRSA strains are always highly resistant to multiple antibiotics [46,57]. The high resistance rate among MRSA towards commonly

used antibiotics (Fig. 2) is of great concern. Today, MRSA isolates often resistant to several antibiotics [58]. Significant resistance was observed in case of Sulfamethoxazole – trimethoprim, erythromycin, Clindamycin, ciprofloxacin, tetracycline and gentamicin. This is probably attributed to prolonged antibiotic usage in animal feeds resulting in enhanced selection pressure [59,60]. Because of the widespread use of antibiotics, especially in developing countries, the resistance profile of MRSA and other microorganisms [61] is changing, evidenced by increasing occurrences of high antibiotic resistance among MRSA populations. Additionally, The high resistance rates observed in this study is consistence with what has been reported in other studies elsewhere [62,63].

Vancomycin is a glycopeptide antibiotic used for the treatment of Gram-positive bacterial infections including MRSA. The appearance of MRSA strains resistant to vancomycin has been perceived as a formidable threat in the therapeutic field, as this antibiotic is traditionally used as a drug of last resort [64]. However, the presence of Vancomycin resistance among of MRSA strains isolated from pigs raises more questions and warrants further investigation.

5. CONCLUSION

There is a very high colonization rate of the swine with *S. aureus* including MRSA which is very resistant to the commonly used human antibiotics. The data from this study showed that MRSA is present in swine population which can serve as a reservoir. The rate of spread of MRSA and its unique ability to acquire antibiotic resistance calls for urgent and well-coordinated surveillance programme to combat this situation.

6. FUNDING

This work was financially funded by German academic exchange (DAAD). Uganda National Health Laboratories – UHNLs was formerly known as Central public health laboratories – CPHL (Ministry of Health – Uganda) and Dr Benon Asiimwe (Associate professor – Makerere University college of health sciences).

COMPETING INTERESTS

There are no competing conflicts of interest concerning this article.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24547



Microbiology Research Journal International

26(2): 1-10, 2018; Article no.MRJI.44939
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Highly Resistant *Staphylococcus aureus* Isolated from Patients Attending a Tertiary Hospital, South Western Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/44939
Editor(s):
(1) Dr. Sabina Fijan, University of Maribor, Slovenia.
(2) Dr. Ana Cláudia Coelho, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Wanpen Chaicumpa, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.
(2) Rania Abozahra, Damanhour University, Egypt.
(3) Devrim Dunndar, Kocaeli University Medical Faculty, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/28143

Original Research Article

Received 20 September 2018
Accepted 03 December 2018
Published 05 January 2019

ABSTRACT

Background: *S. aureus* is a frequent cause of human infections and is one of the most important nosocomial pathogens. Widespread antimicrobial resistance has limited therapeutic choices to treat *S. aureus* infections. Methicillin-resistant *S. aureus* has continued to cause significant infections today challenging public health initiatives to a better healthcare.

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Materials and methods: This was a cross-sectional study carried out between February 2016 and January 2017 among inpatients at Kabale Regional Referral Hospital, Various clinical specimens were collected basing on nature of infection, and analysed using standard phenotypic methods to characterise *S. aureus*. Presence of MecA gene was detected by PCR to confirm methicillin resistance strains. Antibiotic susceptibility tests were done to determine resistance patterns using standard methods.

Results: The study revealed the prevalence of *S. aureus* at 86.7% (n = 390) among inpatients, where; 223(57.2%) were Methicillin-resistant *S. aureus*. Methicillin-resistant *S. aureus* was predominantly isolated from surgical sites; 56.9 %(n = 166), Road traffic accident wounds 67.9% (n = 19) and Burn wounds; 56.3% (n = 09).

Conclusion: There was high prevalence of *S. aureus* among inpatients and majority of the isolates were methicillin-resistant. Methicillin resistant strains were highly resistant to multiple antibiotics that are commonly used.

Keywords: MRSA; inpatients; antibiotic resistance.

1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is the major pathogen responsible for various infections, including bacteraemia, skin and soft tissue (SST) and surgical site infections [1]. The presence of methicillin-resistant *S. aureus* (MRSA) imposes a significant burden on the public health care system due to emergence of antimicrobial resistance (AMR) [2]. S. *aureus* is widespread bacteria freely living in different environmental niches, and to human host, it colonises the axilla, perineum and the anterior nares of the nostrils [3,4]. Skin-to-skin contact from an infected host or colonised individuals forms the basis of common mode of transmission [5]. In the immunocompromised hosts, *S. aureus* infections can be serious and severe leading to increased mortality and morbidity [6,7].

The distribution of MRSA in any given healthcare setting varies depending on hospital wards, locations and hygiene [8]. MRSA remains a public health challenge due to limited availability of therapeutic options to manage infections [9,10], and it is increasingly reported in Uganda [11-14]. Therefore, this study aim to determine the prevalence and antibiotic susceptibility patterns of *S. aureus* isolated from clinical specimens of inpatients admitted at Kabale Regional Referral Hospital (KRRH), a tertiary health care settings located in Kabale District, South Western Uganda.

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross sectional study carried out between, February 2016 to January 2017 onto non repetitive clinical specimens (n = 450) collected from consented inpatients who were admitted in various wards at KRRH.

2.2 Sampling Procedure and Specimen Collection

The inpatients were selected randomly from various wards at KRRH. The study specimens were collected and transported to the laboratory for bacteriological analysis. Specimens were collected from surgical sites, sputum, blood, wound abscess, trauma wounds, burns and Cerebral spinal fluids (CSF) and Urine. Sterile cotton swabs (Fisherbrand ™) were used to collect pus from bed sores other types of wounds. CSF and Knee joint aspirates were appropriately collected in a clean and sterile plain vacutainer universal tubes (BD vacutainer®- yellow top) by clinicians and sent to the laboratory. Sputum samples were collected and received in clean plastic sputum container (Biosigma). Commercially prepared blood culture bottles (BACTEC™ plus Aerobic/F culture vials and BACTEC™ plus anaerobic/F) were used to collect and transport blood for culture from patients suspected with bacteraemia. Urine samples were collected into sterile wide mouth urine containers (SARSTEDT).

2.3 Isolation and Identification of the *S. aureus*

All specimens were processed at KRRH microbiology laboratory. Blood culture bottles were accessioned and incubated in BACTEC ™ FX 40 blood culture system for a maximum incubation period of 168 hours [15]. Pus swabs, urine and sputum samples were separately inoculated into the Mannitol salt broth (MSB)

(HiMedia- India) and incubated aerobically at 37°C for a maximum period of 48 hours. The broth with presumptive *S. aureus* growth was determined by the presence of yellow colour of the culture broth media [16]. Positive broth cultures, as well as positive blood culture vials, were subcultured onto Mannitol salt agar (MSA) media (Oxoid™) and incubated at 37°C± 2 for 24 - 72 hours until appropriate growth was observed [16]. Every new batch of MSB, MSA and blood culture media bottles (Bactec TM plus aerobic/F medium) were quality controlled using *S. aureus* ATCC 6538 as positive control and *Escherichia coli* ATCC8739 as negative control prior to use [17].

S. aureus was phenotypically identified as yellow colonies which were phenotypically identified using biochemical tests that included catalase, coagulase and Staphaurex™ Plus Latex Agglutination Test (Remel™) test [18]. Every isolate that was both catalase, coagulase and Staphaurex™ Plus Latex Agglutination test positive was presumed to be *S. aureus* and subcultured on to the Nutrient agar as a purity plate and incubated aerobically at 37°C± 2 for 24 hours.

2.4 Antimicrobial Drug Susceptibility Testing

All the phenotypically confirmed *S. aureus* isolates were screened for methicillin resistance using cefoxitin (30 µg) disk diffusion method, where a suspension of organism equivalent to 0.5 McFarland turbidity standard were prepared and inoculated onto Mueller-Hinton agar (Oxoid™) by swabbing over agar surface. Cefoxitin 30 µg disk was applied to the plates which were later incubated at 35°C± 2 for 18-24 hours. The zone of inhibition diameter around Cefoxitin disc were measured using a digital Vanier calliper (Fisherbrand™) and results recorded. The results were compared with the Clinical and Laboratory Institute Standard (CLSI, 2015) where any isolate with zone diameter of ≤ 21 mm was labelled as MRSA while those with ≥ 22 mm were labelled as MSSA (CLSI, 2015) [19]. All the phenotypic MRSA and MSSA were inoculated into 30% glycerol in brain heart infusion (BHI) broth (Difco Laboratories) in cryovial tube and stored at -80°C until retrieved for further testing.

All the isolates were tested for susceptibility to selected antibiotics which included; Amoxycilin and clavunate (20/10 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), Imipenem(10 µg), Trimethoprim and sulfamethoxazole (1.25/23.75 µg), Tetracycline (30 µg), Vancomycin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), Rifampicin (5 µg), Linezolid (30 µg), Teicoplanin (30 µg) using Kirby Bauer disk diffusion technique according to CLSI, 2015 guidelines [19]. The *S. aureus* colonies were emulsified in approximately 5mL of sterile normal saline to 0.5 McFarland turbidity standard equivalent of the bacterial suspension. After 15 minutes, a sterile cotton swab was used to inoculate dried Mueller Hinton agar plate by streaking the swab over the entire sterile agar surface repeatedly, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. After 5 minutes, the drug-impregnated disks were applied in no closer than 24 mm from center to center. The plate was incubated at 35± 2°C in ambient air for 18 to 24 hours.

The zones of inhibitions were determined by measuring the size of clear zones using a digital Vanier calliper (Fisherbrand™), comparing the zone sizes within the CLSI, 2015. The results were reported as Resistant (R), Intermediate (I) or Sensitive(S). The zone of clearance with flattening characteristics (D-shaped) around clindamycin in the area between the former and Erythromycin was observed to detect inducible clindamycin resistance. All the phenotypic Vancomycin resistance strains were further tested using Biofire FilmArray for presence of vanA/B gene [20].

Mueller Hinton agar for this study was quality controlled (QC) using S. *aureus* ATCC 25923, *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). In addition, weekly Antibiotic QC was constantly carried out to ensure the quality and validity of the antibiotic susceptibility study results. All the isolates were preserved in 30% glycerol in sterile tryptic soy broth and stored in the biorepository at the Uganda Central Public Health Laboratories (CPHL).

2.5 DNA Extraction and PCR detection of mecA gene

DNA was extracted from all the phenotypic MRSA and MSSA colonies previously stored in 30% glycerol in BHI broth (Difco Laboratories). The isolates were subcultured onto sheep blood agar (SBA) and incubated at 35±2°C for 24 hours to obtain pure colonies. About 1-2 pure colonies of *S. aureus* were suspended in 25 µl of

sterile distilled water and boiled at 100°C in a digital heat block (Thermo scientific™) for 15 minutes [21]. About 05 µl volume was directly used as a template for PCR amplification. The mecA gene was amplified using the primers "MecA F; 5'TCC AAT TAC AAC TTC ACC AGG3' and "MecA R; 5'CCA CTT CAT ATC TTG TAA CG3'"as described by Wang et al. [22] with the Amplicon size approximately 180 bp which were consistent with *mecA* gene amplification. A 50 µl PCR reaction mixture was used which included; 45 µl of master mix containing PCR buffer (x1), dNTP mix (0.2 mM of each), primer (0.5 µM), Taq DNA polymerase (0.25U), and MgCl 2 (1.5 mM) with 5 µL of template DNA. Cycling conditions were set at 94°C as hot start for 4 minutes followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute and final extension step at 72°C for 3 minutes. PCR products were loaded onto 2% agarose gel (Invitrogen, Carlsbad, CA, USA) previously mixed with ethidium bromide gel dye (0.5 µg/mL) and run electrophoresis followed by gel visualisation using UV transilluminator (Fig. 2).

2.6 Data Analysis

The data was entered in EpiData version 3.1.2701.2008, and statistical analysis done by descriptive statistics using SPSS version 23.

2.7 Ethical Considerations

The study was approved by Institutional Review Board of Mbarara University of science and technology (MUST) and Uganda National Council of Science and Technology (UNCST) study Number 13/08–15. The procedures followed were in accordance with the ethical standards of these committees on human experimentation, and with the Helsinki Declaration of 1975 as revised in 2000.

3. RESULTS

A total of four hundred and fifty non-repetitive specimens were collected from 450 inpatients and those from females (n= 241) were slightly higher than those from males (n = 209). The mean age of patients hospitalised was 35.5years and the overall age range was from 5days old to 88years. Specimen were collected from Children; 57(12.7%), teenagers; 71(15.8%), youth; 151(33.6%), middle-aged; 56(12.4%) and the old;113(25.1%). The mean length of hospitalisation was higher in elderly patients compared to other patients age groups (Table 1).

Among all the clinical specimens analysed (n = 450), *S. aureus;* 390(86.7%) was the common bacterial pathogen isolated among which 223(49.6%) were MRSA and 167(37.1%) were MSSA. *S. aureus* was high among surgical site specimens; 74.9%(n =292), which also yielded the highest number of MRSA organisms 166 (74.4%). In general, the MRSA prevalence among the clinical specimens was 49.6% and among the *S. aureus* isolates was 57.2% (Table 2).

MRSA isolates highly resistant to resistant to Amoxicillin - clavunate;100%(n=223), TMP–SMX; 100% (n = 223), Ceftriaxone; 100%(n =220) and Imipenem;100%(n = 49). While MSSA were highly resistant to TMP-SMX; 77.8%(n = 130) and Erythromycin 59.9%(n = 100). Among the 223 of MRSA isolates, 03(1.3%) were vancomycin-resistant (VRSA) confirmed by BioFire FilmArray (Fig. 1).

Table 1. Age characteristic of patients (in years) and mean length of hospitalisation

Age group	Age range (years)	MLH (Hours)	Male (n = 209)	Female (n =241)	Total (n=450)
Child	≤ 12	96	25	32	57(12.7%)
Teenagers	13 – 19	120	33	38	71(15.8%)
Youth	22 – 35	120	73	78	151(33.6%)
Middle age	36 – 45	168	27	31	56(12.4%)
Old	> 45	196	51	62	113(25.1%)

Footnote: n = sample size; MLH =Mean length of hospitalisation; P>.005

Table 2. *S. aureus* profile isolated from various clinical specimens

Type of specimen	Specimen	*S. aureus*	MRSA	MSSA
Surgical site swabs	308	292(74.9%)	166(74.4%)	126(75.4%)
Bed sore swabs	10	07(1.8%)	05(2.2%)	02(1.2%)
Abscess swabs	7	06(1.5%)	02(0.9%)	04(2.4%)
Burn wound swabs	20	16(4.1%)	09(4.0%)	07(4.2%)
Blood culture	12	09(2.3%)	04(1.8%)	05(3.0%)
Sputum	30	11(2.8%)	08(3.6%)	03(1.8%)
Knee joint aspirate.	5	03(0.8%)	02(0.9%)	01(0.6%)
Urine	25	18(0.8%)	08(3.6%)	10(6.0%)
RTA wounds swabs	33	28(7.2%)	19(8.5%)	09(5.4%)
Total specimen and isolates	450	390(86.7%)	223(49.6%)	167(37.1%)

Footnote: n= sample size; RTA = Road traffic accident; MRSA = Methicillin resistant S. aureus; MSSA = Methicillin sensitive S. aureus. P>.005

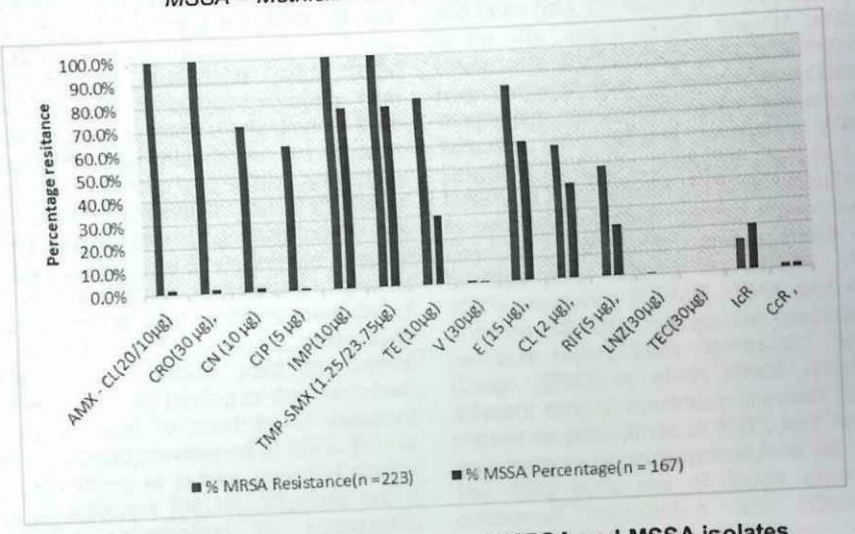


Fig. 1. Antibiotic resistance pattern of MRSA and MSSA isolates

Footnote: AMX –CL = Amoxycilin and clavunate; CRO = Ciprofloxacin; CN = Gentamycin; CIP = Ciprofloxacin; IMP = Imipenem; TMP – SMX = Trimethoprim and sulfamethoxazole TE= Tetracycline; V = Vancomycin; E= Erythromycin; CL = Clindamycin; RIF = Rifampicin; LNZ = Linezolid; TEC = Teicoplanin; MRSA= Methicillin resistance S. aureus; MSSA = Methicillin sensitive S. aureus.

4. DISCUSSION

The prevalence of *S. aureus* was 86.7% among inpatients admitted at KRRH. This is higher than previously described in other Ugandan hospitals; 46% in Mulago National hospital [14], 27% in Mbarara Regional Referral Hospital [13] and 31.6% in neonatal ward at Mulago national hospital [23]. Also, the findings confirm reports of earlier studies implicating *S. aureus* as the common bacterial infections affecting hospitalised patients [24]. Bacterial infection due to *S. aureus* is always associated with increased mortality, morbidity, and length of hospital stay [25]. Potential predisposing risk factors like previous surgeries, severe burns, road traffic accident trauma, the presence of indwelling devices such as catheters, ward overcrowding and prolonged lengthy hospital stays are probable reason for high prevalence at KRRH. Enhanced infection prevention and control strategies is required to reduce the prevalence.

S. aureus infections of the surgical sites was 74.9% which is higher than previously reported in other Ugandan Hospitals [13,29]. Invasive surgical procedures are the common mode of treatment at Surgical and obstetric wards. However, bacterial colonisation of the surgical sites may reveal a glaring lack of adequate postoperative care and failure to maintain sterility during surgical procedure. Postoperative surgical site infections with *S. aureus* remains a significant cause of morbidity in surgically

treated patients that requires additional cost of care [27].

We report frequency of *S. aureus* in urine samples was 0.8% among inpatients especially those with urinary catheter devices. Isolation of *S. aureus* from urine samples probably indicates colonisation. The presence of a urinary catheter is the most important risk factor for bacteriuria which adversely affect patient outcomes [30]. Blood borne *S. aureus* isolates was 2.3% than previously reported in Jimma University Specialized Hospital, Ethiopia and Mulago National hospital, Uganda [31,23]. Previous antibiotic therapy before blood culture is taken could probably suggest presence of low prevalence [30]. Physical trauma due to burns, bed sores (pressure sores) or road traffic accident predisposes *S. aureus* infection. The overall prevalence of *S. aureus* infection of burn wounds was 80% with majority of the isolates being MRSA. This is much higher than previously reported in tertiary care health care facilities of Ethiopia [31] and Ghana [32]. Bed sores are more strongly associated with prolonged hospital stays [33]. The incidence of pressure ulcers in hospitalised patient is often a direct measure of the quality of medical care provided, particularly in the meticulous attention paid to careful positioning and frequent turning of the bedridden patient. Wounds due to road traffic accident (RTA), had *S. aureus* prevalence of 85%. This is consistent with Wong et al who reported similar prevalence in Malaysia [34,35]. Wounds are a risk factor for colonisation by pathogenic bacteria, including multidrug-resistant microorganisms like *S. aureus* particularly MRSA which interferes with wound healing with consequent increase in the severity of the lesions.

The emergence and spread of antibiotic resistance remains a global public health concern. Continuous update about the prevalence and distribution of antibiotic resistance mong MRSA isolates is valuable for clinical and epidemiological intervention. We also report the prevalence of MRSA as 49.6% among hospitalised patients. This is consistent with Chakrakodi et al. in the Tertiary Care Indian Hospital [26]. However, this is higher than is previously reported in S. Korea [28], Australia [8] and India [36]. Probable attributes are suggested to be the inappropriate use of antibiotic or inadequate drug dosage during treatment. It has been argued that, previous hospitalisation is among the contributing factor that results in the selection of multidrug resistance MRSA due selective antibiotic pressure [36]. Presence of MRSA in hospitalised patients indicates a need for greater infection control measures, proper use of antibiotics, and careful inpatient management. Among the MRSA; there was 100% resistance to TMP - SMX, and Amoxicillin – clavunate. The findings are consistent with a previous report from Democratic Republic of Congo (DRC) in which MRSA collected from different clinical specimens showed TMP - SMX resistance prevalence of 100% and Amoxicillin – clavunate resistance prevalence of 89% [37]. The long term use of these antibiotics and concurrent colonisation with other resistant bacteria [36,37] explains emergence of high resistance rates.



Fig. 2. Results of mecA gene PCR where an Amplicon of approximately 180 bp was expected

L is 100 bp ladder,1- Positive control (MRSA ATCC 43300), 2 – negative control (MSSA contains a Methicillin Susceptible S. aureus strain ATCC 25923) Lanes 3, 4,5,6,7,9,11,12,1314 and15 mecA positive (MRSA) from clinical samples of inpatients. Lane 8, 10 and 16 mecA negative (MSSA) isolates from clinical samples. L2 is the Negative amplification control.

The widespread and uncontrolled use of antibiotics undoubtedly accelerates the evolution of *S. aureus*, acquiring multiple resistance genes and therefore capable of surviving in almost all antibiotic families, i.e., beta – lactams, Tetracyclines, fluoroquinolones [38], Macrolides [39] and aminoglycosides [40]. In addition, MRSA isolates resistance to gentamycin was 71.7%. this is higher than 47.8% previously reported in Iran [40]. A resistance rate of 84.5% reported in the Sub Himalayan Region of India [41], reflects and increasing resistance nature of MRSA towards aminoglycosides. Similarly, the report indicates Tetracycline resistance rate of 80.7%, which is much higher than previous reports from Tehran (61%) [42]. Tetracycline is a broad-spectrum antibiotic and have been widely used indiscriminately which probably explain the high resistance rate. Generally, there is significantly higher antibiotic resistance among MRSA strains observed towards the set commonly used antibiotics with exception of Vancomycin, Teicoplanin and Linezolid. MRSA resistance to various antibiotics has increased dramatically at an alarming rate and could probably due to unrestricted use this antibiotic [43,38].

Clindamycin has been an exceptional antibiotic *Staphylococcal* infections and an alternative in penicillin-allergic patients. Its desirable oral bioavailability has been credited as an excellent option for outpatient treatment and changeover after intravenous antibiotics. However, the rate of inducible clindamycin resistance (iCR) (16.2%) requires an urgent response, and has been previously reported elsewhere [44,45]. The consequence of excessive use of macrolides is responsible for raising resistance in this class of antibiotics. However, differences in the resistance rate among regions emphasise the need for generating a local resistance data to guide empirical therapy. Detection of iCR is important for the use of clindamycin in Staphylococcal infections including MRSA.

Treatment of VRSA infections is challenging today often associated with persistent infections, treatment failure, and poor clinical outcomes. However, there is limited literatures regarding VRSA from clinical isolates in Uganda. The current study report of 1.3% resistance rate of MRSA to Vancomycin, is probably the first of its kind to be reported in this region. Disk-diffusion sensitivity systems and automated methods are not very reliable in detecting VRSA [46] and therefore, we confirmed these isolates for the presence of vanA/B gene using Biofire FilmArray. Vancomycin has had perhaps the slowest rate of development of resistance among antibiotics as a glycopeptide antibiotic, inhibits cell wall biosynthesis and is among the drugs of choice for treatment of severe MRSA infections. The molecular basis of resistance in vancomycin resistance *S. aureus* is polygenic and involves stepwise mutations in genes encoding molecules primarily those in cell envelope biosynthesis. The *vanA* gene and operon, which is present on a plasmid is responsible for reduced susceptibility of *S. aureus* toward vancomycin, believed to be transferred from *Enterococcus faecalis* and *E. faecium* [47]. Environmental factors contributing to vancomycin resistance include irrational use of antibiotics; over-the-counter availability without prescriptions; injudicious use in hospitals, agriculture, fisheries and animal husbandry, which could result in increased selective pressure of vancomycin. Finding VRSA isolates in a rural setting represents a serious public health concern. We suggest stringent infection control policies to prevent transmission of such life-threatening isolates in the hospital setting.

There was no MRSA isolates resistance against teicoplanin (Glycopeptides) and Linezolid observed. Walkey et al recommended empirical use of these antibiotics because of their presumed efficacy against MRSA [48]. However, we suggest that such decision should be based routine and regular antimicrobial surveillance, local availability, and local cost. The limitation of this study did not differentiate community acquired and hospital acquired *S. aureus* and MRSA.

5. CONCLUSION

There was high rate of MRSA with multi drug resistance infections among inpatients. This justifies the need to develop a sustainable policy on screening and decolonisation of MRSA from patients as they are admitted to wards and prompt infection control and prevention at the hospital. The high incidence of MRSA and multidrug resistance permits for the judicial use of antibiotics to avoid therapeutic calamity. Vancomycin is no longer taken as excellent antibiotic with activity against clinical isolates of MRSA. We recommend strengthening on -site laboratory capacity to routinely perform antibiotic susceptibilities on all isolates from clinical specimens to detect emerging resistance and spread. This will reduce empirical antibiotic

prescription, facilitates the positive clinical outcome.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article and excel file attached.

ACKNOWLEDGEMENT

This work was supported by the management of Central Public Health Laboratories (CPHL), Ministry of Health, Uganda.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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http://www.sciencedomain.org/review-history/28143