IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* OBTAINED FROM RAW DAIRY MILK AND HUMAN BLOOD

ONGECHI J. OGETO (BPharm)

Department of Public Health, Pharmacology and Toxicology

University of Nairobi

Thesis Submitted in Partial Fulfilment of Requirements for Master of Science Degree in Pharmacology and Toxicology of the University of Nairobi

2021

DECLARATION

Declaration by Student:

I, Ongechi J. Ogeto declare that this thesis is my original work and has not been presented for the award of any other degree in any other institution

Ongechi J Ogeto (BPharm)

Sign _____ Date 19/11/2021

Declaration by Supervisors:

This thesis has been submitted with our approval as the university of Nairobi supervisors

Prof. Simon E. Mitema (BVM, MSc, PhD),

Department of Public Health, Pharmacology and Toxicology

University of Nairobi,

Sign _____ Date_21/11/2021

Dr. Gabriel O. Aboge (BVM, MSc, PhD),

University of Nairobi,

Department of Public Health, Pharmacology & Toxicology

Sign _____ Date 19-11-2021

DEDICATION

I dedicate this work to my dear wife Lilian Kemunto, my children; Audrey N. Ogeto and Amanda M. Ogeto for their prayers, support and encouragement during throughout my academic journey. To my loving mother, who has stood with me in prayers and encouraged me to pursue this study. To my late father James Ongechi, who believed in me and inspired in me to become a scientist with a meaningful vision of human life.

ACKNOWLEDGEMENTS

I wish to acknowledge with enormous gratitude to my supervisors; Prof. Simon E. Mitema of the Department of Public Health, Pharmacology and Toxicology, University of Nairobi for his professional oversight, critiques and inspirations that influenced my writing and completion of this work; Dr. Gabriel O. Aboge of the Department of Public Health, Pharmacology and Toxicology, University of Nairobi whose professional advice in orchestrating the flow and illustrations of this work has been indispensable. I also acknowledge the financial support from Prof. Mitema's research grant that has facilitated this work.

I owe thanks to the dedicated technical staffs in the Department of Public Health, Pharmacology and Toxicology, University of Nairobi for their technical assistance, support and encouragement they extended to me, during my fieldwork, laboratory work and the entire course of this study, in particularly; Dr. Nduhiu Gitahi, Mr. A. O. Mainga, Mr. J.K. Macharia, Mr. L. Bett, Mr. R.K.Rono, Mr. J. Nderitu and G. Pacho.

My gratitude also goes to my postgraduate classmates Dr. N. Mercy, Dr. C. Chebet and Mr. Alex for their friendship, company, useful criticism and exchange of ideas throughout my study. Also I extend my deep appreciation to Miss Margaret Mukami for her devoted secretarial work and organization of this research work.

My verbosity utterly fails to express my love and affection towards my beloved wife Lilian Kemunto, my daughters Audrey and Amanda and my family members, without their cooperation, love, affection and financial support they accorded me, inspired me to scale the heights of education.

I owe much to my beloved sister Veronicah and brother Duke, whose inspiring words and encouragement, financial support has made me sail through rendered my career development.

I highly acknowledge the support I got from the Chairman, Department of Public Health, Pharmacology and Toxicology, Prof. J. M.Mbaria, where the research was carried out.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

BA	: Blood Agar
blaZ	: β-Lactamase gene
Bp	: Base pair
MRSA	: Methicillin Resistant Staphylococcus aureus
CA-MRSA	: Community acquired Methicillin Resistant Staphylococcus aureus
CC	: Clonal complex
Ccr	: Cassette chromosome
DNA	: Deoxy ribonucleic acid
dNTP	: Deoxy ribo nucleoside triphosphate
Fig.	: Figure
G	: Gram
h	: Hour
HA-MRSA	: Hospital acquired Methicillin Resistant Staphylococcus aureus
I.U	: International Units
LA-MRSA	: Livestock associated Methicillin Resistant Staphylococcus aureus
mecA	: Mec A gene
mecC	: Mec C gene
mg	: milli gram
$Mgcl_2$	: Megnesium chloride

MHA	: Muller-Hinton agar
min	: Minutes
Ml	: Millilitre
MLST	: Multilocus sequence type
Mm	: Millimolar
MSA	: Mannitol Salt Agar
Ng	: Nano gram
Nm	: Nano molar
PBP	: Penicillin Binding Protain
PCR	: Polymerase Chain Reaction
Rpm	: Revolution per minute
SCC	: Staphylococcal cassette chromosome
Spa gene	: Staphylococcal protein A
Taq	:Thermus aquaticus
TBE	: Tris -Borate with EDTA
TSA	:Tryptic Soya Agar
UV	: Ultra Violet
µg	: micro gram
µl	: micro litre

ABSTRACT

Staphylococcus aureus (*S. aureus*) is a unique microorganism among *Staphylococcus* spp notoriously recognized globally for its clinical importance in causing clinical or subclinical bovine mastitis in livestock. In humans, it causes food poisoning, toxic shock syndrome, scalded skin disease and bacteraemia as an invasive complication, which may result to osteomyelitis, endocarditis, boils, cellulitis, pneumonia and thrombophlebitis among others. The dissemination of *S. aureus* and its variant methicillin resistant *S. aureus* (MRSA) between different animal species has been documented in many developed countries especially in regions of high dairy farming, pointing out livestock associated MRSA (LA-MRSA), community affiliated MRSA (CA-MRSA) and hospital affiliated MRSA (HA-MRSA) which may freely be transmissible between domesticated and wild animals, poultry and humans.

The aims of this study included: isolation and identification of *S. aureus* from blood of human patients and raw cow milk, determinination of antimicrobial susceptibility (AST) patterns of *S. aureus* from human blood and raw dairy milk from selected farms in peri-urban Nairobi, to determine and compare resistant phenotypes of *S. aureus* strains isolated from milk against those isolated from blood of human patients and to determine the various genetic determinants for MRSA strains and therafter undertake sequencing g of resistant genotypes.

The study used convenience sampling strategy, in a one off sampling process employing inclusion and exclusion criteria. The samples were collected between November 2016 and October2017. A total of 353 milk samples and 142 human blood samples were collected

employing aseptic techniques and transported to the University of Nairobi, Department of Public Health, Pharmacology and Toxicology for *S. aureus* isolation and characterization. Isolation and identification of coagulase positive (COPs) *S. aureus* was done by selective media, namely Mannitol salt agar (MSA) and coagulase testing using reconstituted rabbit plasma and then genotypically confirmed by Polymerase chain reaction (PCR) using specific primers for nuc (thermonuclease) gene of *S. aureus*. AST of *S. aureus* isolates was done by disk diffusion method employing Clinical and Laboratory Standards Institute (CLSI), 2017 guidelines using *S. aureus* ATCC 25923 as standard reference organism. A panel of 8 antibiotics were used for AST; cefoxitin 30µg (as the surrogate antibiotic for methicillin), ampicillin 10µg, gentamycin 10µg, ciprofloxacin 5µg, amoxicillin-clavulanic acid 30µg, erythromycin 15µg, tetracycline 30µg and trimethoprim /sulfamethoxazole 1.25/23.75µg. The diameters of zones of inhibition were measured to closest whole millimetre, then the interpretative criteria for each antimicrobial agent was determined using the criterion described by CLSI, 2017. The isolates that tested resistant phenotypically to cefoxitin 30µg were genotypically identified through amplification of the nuc gene, *mec*A gene and *mec*C gene by PCR specific primer pairs.

PCR assay was employed to detect resistant determinants (*mec*A and *mec*C) genes, which are linked to conferring methicillin resistance. The PCR amplicons were electrophoresed on 1.5 % agarose gel in Tris-acetate-EDTA buffer containing 0.5µg/ml of ethidium bromide using 100 bp DNA ladder. The gels were viewed and documented using UV transilluminator digital camera (Gelmax 125 imager, Cambridge UK) with UVP software interphase computer (Upland CA, USA)

Positive samples identified through amplification of *mec*A gene and *mec*C had their PCR products, alongside their specific primers (both forward and reverse) previously used for identification of the resistant genes with their PCR products were submitted to Humanizing Genomics, Macrogen Europe Laboratory- Netherlands for sequencing. The Basic local alignment search tool (BLAST) of the NCBI Gene bank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to align the PCR product sequences for similarity check after being read by the Gene Runner software.

The BLAST results were employed in confirmation of *S. aureus* isolates harboring the assayed resistant genes, by alignment with the homologue in the gene bank, which were then linked to the host from which the resistant isolate was obtained.

S. aureus prevalence in raw milk and human blood was 7.4 % and 37.3 % respectively. The *S. aureus* isolates from raw milk were resistant to the following panel of antibiotics; cefoxitin, (11.54%), ampicillin, (15.38%), erythromycin, (3.85%), gentamycin, (7.69%), tetracycline, (15.38%) and sulfamethoxazole-trimethoprim (3.84%) and no resistance were noted to amoxicillin/clavulanic acid and ciprofloxacin. Isolates of *S. aureus* from human blood, had cefoxitin resistance marked at 20 (37.74%), ampicillin 27 (50.94%), ciprofloxacin 15 (28.3%), erythromycin 10 (18.87%), gentamycin 15 (28.30%) tetracycline 19 (35.85), amoxicillin-clavulanic acid 15(28.30%) and sulfamethoxazole-trimethoprim 29 (54.72%) while erythromycin, gentamycin, and sulfamethoxazole-trimethoprim showed intermediate resistance of 9 (16.98%), 1(1.89%) and 2 (3.77%) respectively.

Overall MRSA prevalence among confirmed *S.aureus* isolates from cow milk and human blood was 11.54 % and 37.74 % respectively. All the three *S. aureus* isolates from milk that were phenotypically methicillin resistance did not expressed *mec*A or *mec*C genes by PCR assay while *S. aureus* isolates (n=20) from human blood, 17 (85 %) expressed *mec*A gene and 3 (15 %) isolates did not express *mec*A nor *mec*C genes and 22.64 % expressed *gyr*A gene, 24.53 % expressed *gyr*B gene and 18.87 % expressed *tet*M gene by PCR. On the other hand, 4 (15.38 %) of the 26 isolates from raw cow milk expressed *tet*M gene and the three isolates of *S. aureus* that were phenotypically methicillin resistant did not express *mec*A nor *mec*C genes by conventional PCR assay. This study noted an overall difference in resistance of *S. aureus* strains from humans to nearly all-antimicrobial classes as compared to *S. aureus* isolates from raw milk.

Multidrug resistance was observed among 19 (35.84%) isolates of *S. aureus* from human blood. The most frequent MDR phenotypes for *S. aureus* identified in this study were; FOX-AMP-CIP-GENT-AMC-SXT and FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT in 8(15.09%) and 4 (7.55%) of isolates respectively (Table 4.6). In total, 9 (16.98%) MDR phenotypes were identified; FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT (7.55%), FOX-AMP-CIP-ERY-GENT-AMC-SXT (1.88%), FOX-AMP-CIP-GENT-AMC-SXT (15.09%), FOX-AMP-CIP-ERY-GENT-AMC (1.88%), FOX-AMP-CIP-GENT-AMC (1.88%), FOX-AMP-SXT (1.88%), AMP-TET-SXT (1.88%), AMP-ERY-SXT (1.88%) and ERY-TET-SXT (1.88%). Different resistant phenotypes and their corresponding genetic determinants for resistance of the MDR-*S. aureus* isolates from human blood were detected in this study. About (78.95 %) of the multidrug resistant isolates from human blood were MRSA and 73.68 % of MDR-*S. aureus* harbored *mec*A, 63.16 % *gyr*A, 68.42 % *gyr*B and 26.32 % *tet*M.

Our results indicate that *mec*A gene was the predominant genetic determinant for methicillin resistance phenotypes, followed by *gyr*B, *gyr*A and *tet*M for the resistance of ciprofloxacin and tetracycline respectively. There was no multidrug resistance (MDR) noted among the isolates of *S. aureus* from raw milk but seven resistant phenotypes were evident; TET (7.69%), FOX-GENT (3.85%), AMP-TET (3.84%), ERY- SXT (3.84%), AMP (11.54%), FOX (7.69%), and GENT-TET (3.85%) wherein; AMP and FOX and TET were the top three frequently identified phenotypes.

In this study, *S. aureus* was identified to be present in both dairy milk and human blood. Ciprofloxacin, amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole were the most effective agents against *S. aureus* isolates from cattle while ciprofloxacin, erythromycin and amoxicillin/clavulanic acid were the most effective against *S. aureus* isolates from humans. The study further shows that low to moderate MRSA phenotypes were observed in both cattle and humans, however MRSA strains from human isolates were three folds more than that from cattle. Also the study shows that MRSA isolates from humans harboured *mec*A gene while the isolates from cattle did not express *mec*A nor *mec*C genes. The resistant determinant *mec*A gene in human blood in the current study was alike to some strains of MRSA from animals in other parts of the world and therefore demonstrating the zoonotic pontential of this resistant gene.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Staphylococcus aureus (*S.aureus*) is a primal agent belonging to *Staphylococcaceae* family group of organisms (CABI, 2020). The bacterium co-exists as a normal flora on different parts of the body including; pharynx, skin, intestine and vagina (Lowy, 1998). The micro-organism (*S. aureus*) uses complex regulatory mechanisms to sense varied stimuli to favorable conditions for growth and multiplication, pathogenicity and modulation of its virulence (Balasubramanian *et al*., 2017). It also possesses multiple toxins and virulent mechanisms (Lowy, 1998; Boswihi and Udo, 2018).

The pathogenicity of *S. aureus* is enhanced through secretion of toxins like Panton-Valentine Leucocidin (PVL), 33-kd protein-alpha toxins, and exfoliatin A and B toxins (Lowy, 1998). These toxins pose a health threat to both humans and animals by causing various diseases of the skin including boils, folliculitis, carbuncles, impetigo and other related health tortuousness including toxic shock syndrome (TSS), mastitis and meningitis (Makgotho, 2009).

The bacterium is notoriously known to cause hospital and community invasive and soft tissue infections (Omuse *et al*., 2014; Boswihi and Udo, 2018). The organism is known to be sporadic in a wide compass of ecological habitats and specific parts of domestic animals like dog nose and also from blood and body surfaces of humans (Mbogori *et al.*, 2013). In humans, the organism is responsible for a spectrum of diseases including osteomyelitis, bacteraemia, endocarditis, boils, skin abscesses, cellulitis and surgical site infections and also causes mastitis and septicaemia in dairy cattle, and arthritis in poultry (Mbogori *et al*, 2013).

Other species of *Staphylococcus* such as *S. epidermidis* is linked to causing infections related with indwelling medical devices, *S. saprophyticus* causes infections of the urinary tract system amongst young girls of adolescent age, whereas *S. warneri, S. lugdunensis, S. schleiferi, S. intermedius* and *S. haemolyticus* are inconsistently associated with health care setting pathogenicity (Makgotho, 2009).

Among the Staphylococcus spp., coagulase-positive (COPs) *S. aureus* and coagulase-negative (CNS) such as *S. epidermidis* and *S. haemolyticus* are of human and veterinary medical significance (Misic, *et al*., 2015; Bierowiec *et al*., 2019)

S. aureus is an important clinical pathogen due to its extracellular virulency that enhances its colonisation and pathogenicity after surpassing the host defence mechanism (Bien *et al*., 2011; Balasubramanian *et al*., 2017). Therapeutic management of diseases caused by *S. aureus* has become complicated to health care providers in attaining the intended outcomes due to the pathogen's ability to develop multi-drug resistance (Lowy, 1998; Gnanamani, 2017; Gheorghe *et al.,* 2019).

Prior to 1942, management of diseases caused by *S. aureus* involved the use of β-lactam antibiotics such as penicillins (Makgotho, 2009). After the development of methicillin (semi synthetic penicillins) in the late 1950s and its introduction into clinical practice in 1959, (Chamber and Deleo, 2009; Lakhundi and Zhang, 2018) to manage diseases caused by *S.aureus* as an alternative therapy to natural penicillins (Lowy, 2003), strain of methicillin resistant *S. aureus* (MRSA) was detected in 1961, two years later, following unveilling of methicillin into market (Chambers, 2001) and in the late 1960s, 80 % of community and

hospital affilliated *S. aureus* were reported to be resistant to to penicillin (Lowy, 2003) after which numerous virulent multidrug resistant strains of *S. aureus* were evident,in UK (Jevons, 1961; Livermore, 2000; Lowy, 2003). After UK, MRSA isolates were reported from other parts of European countries as well as Africa viz. USA, Malaysia, Australia, North Africa and East Africa as well (Lakhundi and Zhang, 2018; Guo, *et al*., 2020).

In a decade after MRSA was reported (1961 to 1970), infections related to the pathogenic strain were confined to hospital (Lakhundi and Zhang, 2018) and currently predominantly established in the community (Lowy, 2003). In the early 1980s, the bacterium was noted as the leading causative agent for nosocomial infections (Makgotho, 2009; Lakhundi and Zhang, 2018) and a pattern of inter-transmission between hospital and community MRSA was noted (Makgotho, 2009).

Between 1993 and 2003, new MRSA strains phenotypically and genotypically distinctive from the native hospital acquired MRSA (HA-MRSA) was noted in community indicating phylogenesis of the native MRSA (Makgotho, 2009) and between 1960s and 2005, about 19,000 deaths directly linked to MRSA and 100,000 seriously ill of MRSA infections were reported worldwide (Egege *et al*., 2020). From the year 1987, community associated MRSA (CA-MRSA) increased tremendously causing clinical manifestions such as necrotising severe skin and soft tissue infections, pneumonia, and mastitis (Makgotho, 2009) until 1990s when HA-MRSA became pandemic (Johnson *et al*., 2005) with a doubled hospitalization as aresult of MRSA related infection between 1999 and 2005 (Egege *et al*., 2020). MRSA is a genetically distinctive strain *S. aureus* that expresses *mec*A gene or *mec*C gene and therefore conferring other resistance mechanisms such as change of affinity to penicillin-binding proteins for β-

lactam antibiotics such the penicillins, carbapenems, monobactams and cephalosporins (Gunawardena *et al.,* 2012; CLSI, 2017).

The morbidity and/or mortality related to *S. aureus* infections are very high and in this case, hospital acquired infections are more often related to antibiotic resistant pathogen (Okon *et al.*, 2011). Even though wide range of antibiotics are available in medicine, researchers are still actively searching for a new antimicrobial agent with superior activity due to ability of bacteria becoming resistant to currently available antibiotics (Basak *et al*., 2015), for example 60 % of *S. aureus* strains are methicillin resistant, and some strains have also started becoming resistant to vancomycin (Patrick, 2013; Basak *et al.,* 2015). Currently, the rising incidence of *S. aureus* resistance to vancomycin has spurred fear among the health care providers and this has become growing worldwide concern (Tiemersma *et al*., 2004).

MRSA contributes to a greater percentage of hospital and community acquired infections globally. It is estimated that in European Union over 15,000 people suffer from hospital acquired MRSA related infections and this has increased the burden of In-hospital costs of up to Euro 380 million and longer days of hospitalization (Köck *et al*, 2010). The emergence of multi-drug resistant bacterial strains in many healthcare systems that has narrowed the spectrum of effective antibiotic for clinically challenging infections has become a de-facto monopoly globally for premature deaths and extra days of hospitalization (Peters *et al*., 2019). Overall MRSA accounts for 44 % of heath-associated infections, 22 % of which are attributed to extra deaths and 40 % of extra days of hospitalization due to resistant pathogenic MRSA strain (Kot *et al*., 2020). Community acquired methicillin resistant *S. aureus* (CA-MRSA) strains has emerged to be a principal health concern globally since the late 1980's when it was first reported in western Australia among communities with no previous records of

hospitalization and currently documented evidence rests on colonized animals being reservoirs and shedders and possible transmission between human and animal species may not be ruled out (Boswihi and Udo, 2018). The carrier rate of *S.aureus* amongst healthy individual lies between 15 % and 35 % with 38 % risk of that individual developing an associated infection with an additional infection risk of 3 % when colonized with MSSA (File, 2008).

According to Dora (2011) MRSA prevalence rate in Europe stands at 26 % while in USA, 61.8 % of the patients were colonized by MRSA strains and 38.2 % infected by MRSA related diseases (Jarvis *et al*., 2012). In Asia, *S. aureus* prevalence was noted to be above 60 % of which MRSA contributed for 25.5 % of all the community associated illnesses and 67.4% of hospital related sicknesses (Song *et al.,* 2011). In Africa, data on MRSA is sparingly documented, however *S. aureus* prevalence rates vary from 5 % to 45 % (Dora, 2011) while Omuse *et al.,* (2014) reported MRSA prevalence rates as low as 4 % and as high as 82 %. With the exception of Southwest parts of Africa, MRSA has been reported from most parts of African continent starting with Madagascar with MRSA prevalence rate of 5 %, Algeria 45 %, Tunisia 8.1 % respectively (Dora, 2011).

Among the East African countries, the MRSA prevalence ranges between 16 % to 27 % in which Tanzania reports a prevalence rate of 16 % and Kenya at a prevalence rate of 6.9 % (Aiken *et al*., 2014), However another survey conducted between 1996 and 1997 in eight African countries reported a varied reports of MRSA prevalence rates ranging from 20 % to 30 % in Cameroon, Nigeria and Kenya but the prevalence rate in Tunisia and Algeria was less than 10% (Kesah *et al*., 2003). Bloodstream isolates of *S. aureus* at University Hospital in Kenya retrospectively reported MRSA prevalence rate of 21 % (Omuse *et al*., 2014).

In livestock, MRSA has become a disturbing concern because of its zoonotic nature, contamination of milk or dairy produts and it is associated high disease treatment costs (Keyvan *et al*., 2020) or rather serve as potential reservoirs for zoonotic infections (Fitzgerald, 2012). Various publications from different parts of the world have reported varied prevalence of MRSA in milk as in Iran 16.2 %, (Jamali *et al*., 2015), Italy 2.5 %, (Parisi *et al*., 2016), Italy 0.7 %, (Giacinti *et al*., 2017), Czech Republic 6.1 %, (Klimešová *et al*., 2017), Uganda 56.1 %, (Asiimwe *et al*., 2017) and Turkey 1.7 % (Ektik *et al.,* 2017). In South Africa, 6% was reported in dairy milk in two commercial farms (Ateba *et al.,* 2010) and in Nigeria, Omoshaba *et al*., (2020) reported MRSA prevalence of 18.5 % in raw milk, 37.7 % in sheep, 23.4 % in goats and 7.5 % from nasal swabs of small ruminants. Animals can transmit MRSA resistant strain not only holizontaly or vertically but also in raw dairy products intended for commercial processing and for consumption (Klimešová et al., 2017; Omoshaba *et al*., 2020)

1.2 Statement of the problem

While there is a wide range of antimicrobial agents available in medicine with improved antibacterial spectrum, there remains a worrying clinical concern over the continued possibility of bacteria to acquire resistance to these agents. Even though articles published on MRSA in Africa have reported various possible origins of MRSA strains (animals, animal products and humans); but these MRSA isolates from different independent setting of study have rarely been compared.

The incidences of epidemic strains of MRSA are increasing in many African countries and this situation is posing a feasible threat to available therapeutic agents and alternative options.

In Africa, including Kenya, the prevalence of MRSA clones is not well documented and therefore determining the antimicrobial resistance patterns may address this gap and challenge (Falagas *et al.,* 2013).

1.3. Justification

Elucidating the prevalence of MRSA isolates among animal and human and assessing the phenotypic and genotypic characteristics of MRSA from human clinical isolates and raw cow milk from different regional settings, remains an important step towards curbing inter and cross transmission and upward trends in MRSA related infections in both humans and animals.

Healthcare policy makers on appropriate therapeutic interventions to clinically challenging conditions can use data on MRSA prevalence, generated from this study and facilitate national planning on attainable treatment protocols on common staphylococcal related infections so that an appropriate antimicrobial therapy is initiated for better therapeutic outcomes, with minimal attributable in-hospital costs.

Data on MRSA prevalence in raw milk from this study will help relevant professional body and authorities responsible for surveillance and prevention of bacterial resistance to antimicrobial agents to formulate policies and guidelines emphasizing on areas vulnerable to indiscriminate use of antibiotibics, considerable overlap between antibiotic agents consumed in human or veterinary medicine and overall consumption of antibiotics in animal production and implement antibiotic stewardship to protect animals and humans from the rising threat of antibiotic resistance.

1.4 Hypothesis

MRSA is not common in human blood and raw cow milk.

1.5. Objectives

a). General objective

To identify and determine molecular characterization of Methicillin Resistance *S. aureus* obtained from blood of human patients from Nairobi healthcare facilities at Mukuru slum and raw cow milk from selected farms in Peri-urban Nairobi**.**

b). Specific objectives

I. To isolate and identify the prevalence of *S. aureus* from blood of human patients and raw cow milk.

II. To determine the antimicrobial susceptibility profiles of *S. aureus* isolated from blood of human patients at community healthcare facilities and raw cow milk from selected dairy farms in peri-urban Nairobi, Kenya.

III. To determine and compare antimicrobial resistant phenotypes of *S.aureus* isolated from blood of human patients at community healthcare facilities with that isolated from raw milk from selected farms in peri-urban Nairobi, Kenya.

IV. To determine the genetic determinants responsible for MRSA strains.

CHAPTER TWO: LITERATURE REVIEW

2.0 Description and Taxonomy of *Staphylococcus aureus*

S. aureus is a non-motile, catalase positive, coagulase positive and Gram positive bacterium belonging to the family of *Staphylococcaceae*, with a characteristic golden-yellow pigmentation of colonies (**Fig. 2.1**) commonly arranged in grape-like irregular clusters on solid media culture (Stapleton and Taylor, 2002; Ryan and Ray, 2004). The organism is non-spore forming, facultative anaerobe, which can form lactic acid from the fermentation of carbohydrates (Lowy, 1998; Waldvogel, 2000).

The bacterium grow on varied ecological habitats of either dry condition or conditions of high salt concentration but they favourably vegetate well in high osmotic pressure and low moisture environmental habitats and these adaptive features enables the bacterium to grow and survive in different environments including both in humans and animals (Ryan and Ray, 2004). The bacterium size ranges from 0.5μm to 1.5μm in diameter (Stapleton and Taylor, 2002).

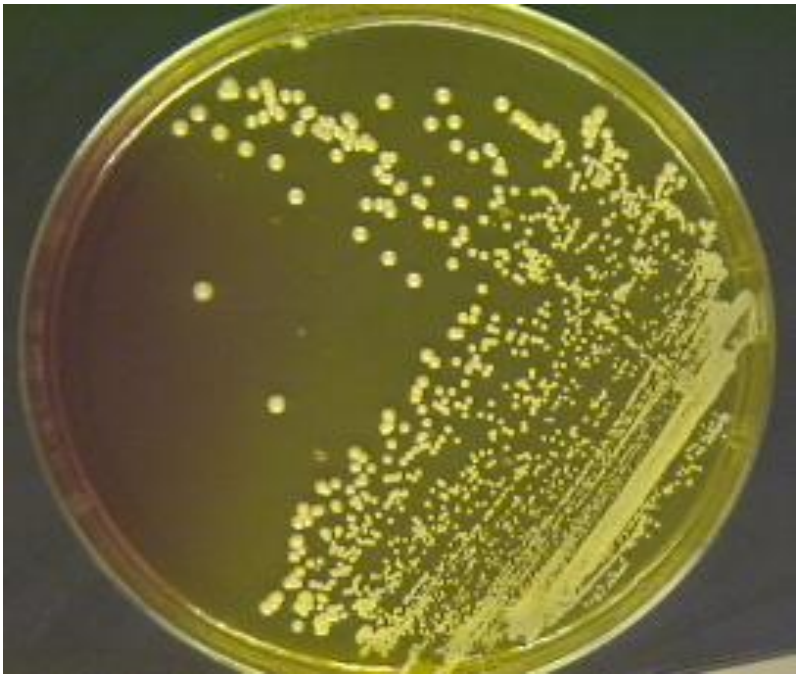


Fig. 2.1 Golden- yellow colonies of *S. aureus* on Mannitol Salt Agar.
Sourse; (https://medchrome.com/wp-content/uploads/2010/05/s.aureus-agar.jpg

The colonies of the microorganism are noticeable with a smooth, convex shape with gold pigmentation when cultured on Mannitol salt agar (**Fig.2.1**) at temperature of 20 ^{0}C to 25 ^{0}C of (Lowy, 1998) and ß-haemolysis on sheep blood agar (**Fig.2.2**) when incubated at 37 ^{0}C for 18 - 24 hours (Zhu *et al*., 2016).

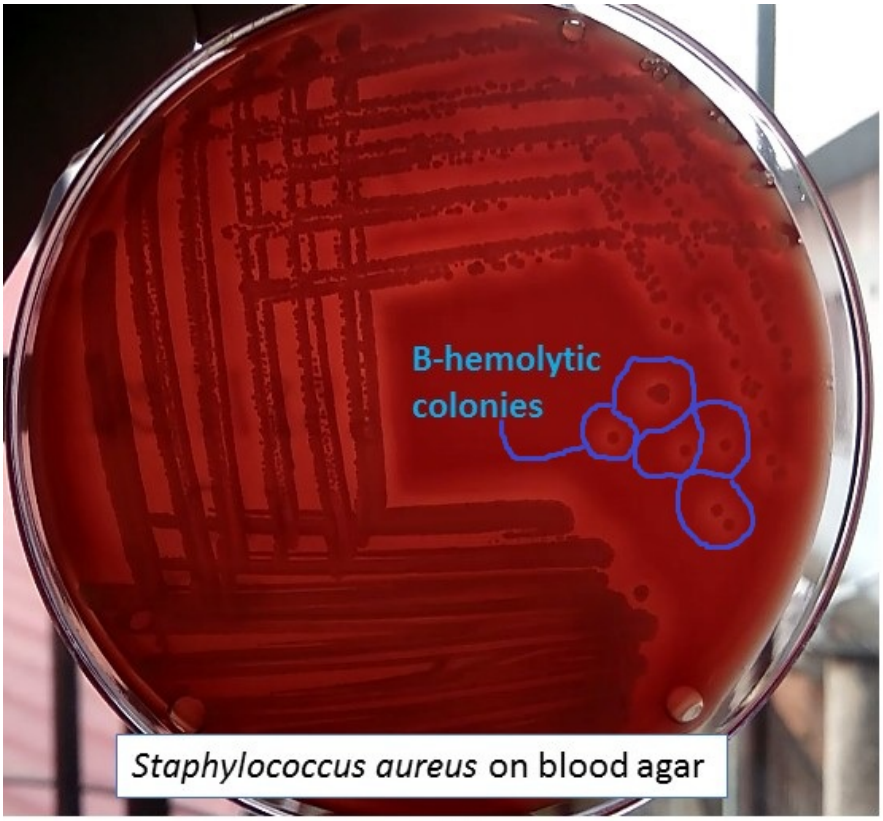


Fig. 2.2 *S. aureus* on a blood agar plate
Sourse;(https://universe84a.com/wp-content/uploads/2019/07/Beta-hemolytic-colony-of-Staphylococcus-aureus-on-Blood-agar.jpg

Table 2.1 Summary of the classification of *Staphylococcus aureus*

Domain	*Bacteria*
Kingdom	*Eubacteria*
Phylum	*Firmicutes*
Class	*Bacilli*
Order	*Bacllales*
Family	*Staphylococcaceae*
Class	*Staphylococcus*

Species (Only of clinical importance to humans)	*S.aureus* *S.epidermidis* *S.saprophyticus* *S.haemolyticus* *S.lugdunensis*

Sourse; (http://www.textbookofbacteriology.com)

There are 45 species under the family *Staphylococcaceae* of which 17 species have been isolated from humans (Bonesso *et al*., 2011). The genus *Staphylococcus* is classified into two major categories namely; coagulase positive (CoPs) such as *S.aureus*, *S. hyicus*, *S. intermedius*, *S. schleifer, S. lutrae,* and *S. dolphin* and coagulase negative (CNP) including *S. epidrmidis*, *S. haemolyticus*, and *S. saprophyticus* which oftenly cause human ailments, the rest of coagulase negative *staphylococcus* are merely opportunistic pathogens with significant potential to cause infections to man and animals (Layer *et al*., 2006). Among the CoPs, *S. aureus* in one of the most infectious species both to man and animals, but the other four species can colonize man but the chances of causing disease are minimal (Bannerman, 2003).

Staphylococci implicates a sprectrum of diseases and sicknesses in human and animals and its pathogenicity is predominantly related to a multi factorial approach including a combination of invasive capacity, antibiotic resistance, and toxin-mediated virulence (Reddy *et al.,* 2017).

2.1 Distribution of *Staphylococcus aureus*

S. aureus is broadly spread within the natural habitat even though they are often found among living organism as colonisers of the mucus membrane, the skin and glands (Grundmann, *et al*., 2010). The bacterium is also a common contaminant of food and can cause food poisoning

(Grundmann, *et al*., 2010; Mbogori, 2016). A report published by Chambers and DeLeo (2009) points out *S.aureus* as the predorminant cause of food poisoning in USA and approximately 2 million cases of nosocomial infections, 230,000 cases of these are caused by *S. aureus* (Bannerman, 2003).

Ten years after the Second World War, there emerged regular cluster of human mastitis and subsequently infections in infant on one end and outbreaks of cow mastitis affiliated with mechanical milking and of infections in industrially reared chicken on the side resulted to the question of reciprocal *S. aureus* transmission between animals and humans (Cuny *et al.,* 2010).

2.2. Infections caused by *S. aureus* in animals

S. aureus is one of the microorganisms of clinical importance in causing mastitis in livestock (Sağlam *et al*., 2017; Boswihi and Udo, 2018), so especially in lactating animals worldwide (Igbinosa *et al*., 2016).

In veterinary medicine, *S.aureus* is attributed to about 64.95 % of cases of clinical or subclinical mastitis (Sağlam *et al.,* 2017) and this has resulted to un-imaginable economic loss in food production industry (D'amico and Donnelly, 2011; Boswihi and Udo, 2018). *Staphylococcus* mastitis has a key role in commercial and home based food health and safety due to the possibility of lactating animals with subclinical or clinical mastitis casting the bacterium into milk meant for either direct consumption or for industrial processing of dairy products (D'amico and Donnelly, 2011). In poultry, the bacterium can also cause septicaemia and arthritis (Mbogori, 2016).

Sources for food contamination more especially of animal origin may be humans or livestock that are engaged in animal husbandry or food processing (Sergelidis *et al.,* 2017). Under conducive environment for vegetative proliferation and secretion of enterotoxin, the isolates of *S. aureus* present in food results to staphylococcal food poisoning (SFP) (Sergelidis *et al.,* 2017) resulting to gastroenteritis that clinically present with vomiting and diarrhoea (D'amico and Donnelly, 2011).

2.3. Infections caused by *S. aureus* in humans.

S.aureus exists as a common normal flora on mucosal membranes, the skin, nasopharynx and anterior nasal nares (Kluytmans *et al.,* 1997). Infections related to this bacterium are as consequence of colonization with the colonizing pathogenic strain to the host cells and access through a previously intact surface after injury or attrition of the mucosal membrane or the skin by the aid of bacterial toxins and exoproteins (Shi *et al*., 2016). It's estimated that 30 % of health humans are intermittent harbours of *S. aureus* and about 20 % of humans are permanent carriers of the bacterium (Omwenga *et al.,* 2019).

Gradually *S. aureus* has adopted and advanced a variety of virulence factors that are prudent in the production and secretion of toxins such as delta and enzymes like alpha and gamma, which are essential mediators of host cell destruction (Otto, 2014).

The microorganism secretes very potent exotoxins such as exfoliatin and enterotoxins including the production of biofilm whim are important for causing various diseases (Otto, 2014).

In human, *S. aureus* is of clinical significance in causing diseases such as osteomyelitis, bacteraemia, skin and soft tissue infection (SSTIs) and pneumonia (Tong *et al.,* 2015). The bacterium is well accommodative to selective antimicrobial pressure and therefore capable of colonizing healthy individual thus acting as a reservoir of infection (Chambers & DeLeo, 2009). *S. aureus* stands to be a frequent causative agent for toxin-mediated diseases when the toxins it produces are ingested via contaminated food Boswihi and Udo (2018). Because of adaptation ability to different environmental conditions, intrinsic virulence and the capability to cause an array of life threatening illnesses, *S. aureus* is possibly the greatest bacterium of concern to human health (Pournajaf *at al*., 2014).

Toxic shock syndrome is one of toxin mediated diseases which may remain relatively localized but the toxin may cause localized and systemic effects such as flu-like symptoms, diarrhoea, abdominal pain, rash fever, hypotension, myalgia, confusion, vomiting and in chronic cases multiple organ failure (Kluytmans *et al.,* 1997; Boswihi and Udo, 2018).

In the case of Ritterson Ritterschein disease in newborn also known as staphylococcal scalded skin (SSS) syndrome which is a common illness in children but rare in adults, the exfoliatin toxin (ET) results to massive sloughing of epidermis and eventually generalized body rash and blisters of the skin with collagen degradation (Otto, 2014; Boswihi and Udo, 2018). Bacteraemia is another systemic complications related to *S.aureus* invasion into the body either secondary to surgical infection, ventilator-associated pneumonia, primary wound infection and catheters or intravenous devices associated infections (Chambers, 2005). The pathogen can further invade the bones resulting to osteomyelitis or may infect the joint spaces to cause septic arthritis and further if it infects the veins it can result to thrombophlebitis (Aires de Sousa and

de Lencastre, 2004). Food contaminated with heat stable staphylococcal enterotoxins causes staphylococcal food poisoning (Boswihi and Udo, 2018; Omwenga *et al*., 2019).

S.aureus remains a confirmed bacterium widely responsible for causing food-borne infections in humans as a result of consuming food products contaminated with preformed heat stable *S. aureus* enterotoxins which is a significant virulence factor among staphylococcal species (Omwenga et al., 2019).

2.4. Methicillin Resistant *Staphylococcus aureus* (MRSA)

MRSA refers to distinctive prototype strain of *S. aureus* that has developed multi-resistance to βeta-lactam class of antibiotics and their derivatives (Pantosti and Venditti, 2009), including cephalosporins, carbapenems and equally with high potential risk of extending resistance to macrolides, aminoglycosides and quinolone (Adhikari *et al*., 2017).

MRSA related infections poses pressure on health systems and is positively correlated to increment in morbidity and mortality rates as rated to other pathogenic microoganisms (Dadashi *et al*., 2018; Elhassan*et al*., 2015). The uptrend in the cases of MRSA infections has become a significant health burden globally (Dadashi *et al*., 2018).

MRSA emerged in UK in 1960, soon after ushering of methicillin (a semi synthetic derivative of penicillin) into the market in the late 1950's as an inhibitor of bacterial cell wall synthesis (Falagas *et al*., 2013; Boswihi and Udo (2018). MRSA currently is the most frequently recognized anti-microbial resistant pathogen globally including east Asia, north Africa, the middle east, the Americas and Europe and Netherlands where MRSA prevalence was

previously minimal with moderate stability for decades, but now have started experiencing an increase in MRSA prevalence rates (Grundmann *et al.,* 2006).

Even though MRSA related infections are of nosocomial origin and attest themselves as complications secondary to colonization, underlying disorder or health care procedures as opposed to representation of defined nosological entities, the negative economic impact as a result of loss of productivity, morbidity and mortality resulting from MRSA cannot be measured easily (Grundmann *et al*., 2006). MRSA and its prototype has attracted worldwide worry due to its consequential effects such as high medical bill, long days of in-patient hospitalization, complications in patient treatment and overall challenge in appropriate choice of therapeutic agent (Okon *et al*., 2011). It's approximated that the financial burdens in terms of treatment of hospital acquired MRSA invasive infections in US hospital to be $25000 as compared to non-MRSA hospital acquired infection, which range at $13973 (Okon *et al*., 2011).

Methicillin resistant occurs as a result of MRSA strains acquiring a mobile staphylococcal chromosomal cassette, which harbours *mec*A, or *mec*C gene, that encode for an altered Penicillin Binding Protein (PBP) known as Penicillin Binding Protein 2a (PBP 2a) subtype which is absent in other susceptible *S. aureus*, resulting to lowered affinity to βeta-lactam antibiotics and its congeners including carbapenems (Petinaki *et al*., 2001; Hiramatsu *et al*., 2013). The elaboration of *mec*A gene and the resultant production of PBP2a is controlled by proteins which encode penicillinase –associated blaR1-bla1 inducer repressor system and tmecRI-mecI as the responding genetic elements (Pitinaki *et al*., 2001). The mecRI gene

encodes for sensing trans-membrane signalling protein for β-lactam antibiotics while mecI is for a repressor protein (Petinaki *et al.,* 2001).

Evaluation of whole gene sequencing, mutations of the endogenous penicillin binding proteins 1, 2 and 3 in *mec*A (Ba *et al*., 2014) there is *mec*C gene which is a homolog of mecA, shares up to 70 % nucleotide similarity with *mec*A (Ballhausen *et al*., 2014) consequently MRSA isolates from humans and animals hosts horboring *mec*C has been postulated to be possible alternative mechanisms for conferring resistance of MRSA strains to βeta lactam antibiotics (Paterson *et al*., 2014**).** Specific alterations in different amino acids existing in PBP cascade (PBP1, 2 and 3) may equally be the groundwork of resistance (Elhassan *et al*., 2015).

Classification of MRSA clones is guided by the sequence type (ST) and the kind of SCCmec type (Boswihi and Udo, 2018). Presently there are four main *mec* elements with their complete structure that have been elucidated currently known as staphylococcal chromosomal cassette (*SCCmec*), and this includes Type I (34kb) which is among the first MRSA strain isolated in UK in 1961(Oliveira and de Lencastre, 2002). Type II consisted of 52kb was identified in MRSA strain isolated in Japan in 1982 and type III with 66kb was isolated from MRSA strain in New Zealand in 1985 (Oliveira and de Lencastre, 2002). Type IV *SCCmec* element which is clinically more problematic consists 20kb to 24 kb and has a structure similar to Type I element in its overall downstream region and *mec*A complex was identified more recently in community acquired MRSA strain (Oliveira and de Lencastre, 2002).

2.4.1 Types of MRSA

2.4.1.1 Hospital acquired MRSA (HA-MRSA)

HA- MRSA contagion is predominantly associated with people who frequented healthcare setting (David *et al*., 2008). Emergence and distribution of HA-MRSA is dependent on various risk factors that are typically favourable for the nosocomial environment and this may include acquisition of HA-MRSA strain during the previous dwelling in a nosocomial setting and the features of HA-MRSA infection only becoming evident at hospital admission (Cuny *et al*., 2010).

A large proportion of typical HA-MRSA strains possess *SCCmec* I, II, or III and therefore based on multi-locus sequencing type they are categorized into sequence type called as New York/Japan (ST_5/*SCCmec*II), ST36/*SCCmec* II (EMRSA-16) and ST22/*SCCmec* IV (Epidemic MRSA) and among the HA-MRSA lineage they are most prevalent (Sato *et al*., 2017). *SCCmec* I harbours mecA gene as the sole dominant for resistance to β-lactams while SCC type II and III have several resistance determinants to non β-lactam class of antibiotics and therefore confer multidrug resistance phenomenon exhibited among the nosocomial MRSA isolates (David *et al*., 2008; Kateete *et al*., 2019).

Genetically, *SCCmec* types I, II and III of HA-MRSA, tend to show multidrug resistance patterns to a wide class of anti- microbial and equally they multiply slowly on culture media (Boswihi and Udo, 2018).

Healthcare related risks due to HA-MRSA infections include medical devices related infections, respiratory infections, surgical wounds infections, infections of the urethral system and other systemic diseases (Boswihi and Udo, 2018). Acquiring HA-MRSA infections is

secondary to compromised immunity, prolonged exposure to multiple broad-spectrum antibiotics, and previous long period of hospital admission including pediatric/old age (Boswihi and Udo, 2018)

2.4.1.2 Community acquired MRSA (CA-MRSA)

MRSA strains that have extensively disseminated among individuals within the community setup without any track of previous hospitalization or contact with healthcare providers were designated as CA-MRSA (Deurenberg and Stobberingh, 2009; Boswihi and Udo, 2018).

Strains of CA-MRSA were firstly recognized and reported in 1980s among healthy individual in Western Australia communities without any record of previous hospital admission (Carvalho *et al.*, 2010; Boswihi and Udo, 2018). Community acquired MRSA strains have a wide distribution of *SCCmec* element type IV, V and VII and appears to harbor exotoxin PVL which proportionately determines the severity of infection (Funaki *at al*., 2019).

Strains of CA-MRSA have majorly been noted to cause potentially fatal illness due to the production of toxin (pvl) with resultant to infections such as necrotizing fascilitis, septic thrombophlebitis, septic arthritis, bacteremia, post influenza pneumonia and hemorrhagic pneumonia (Dufour *et al*., 2002; Boswihi and Udo, 2018).

According to Valsesia *et al*., (2010), strains of CA-MRSA carry small *SCCmec* type IV and tend to grow at a faster rate consequently leading to high infection pressure as compared to strains of nosocomial MRSA. *SCCmec* type IV is relatively more common among

Staphylococcus epidermidis and through horizontal gene transfer mechanisms; *S. aureus* acquire the pathogenic *SCCmec* type IV strain (Bonesso *et al*., 2011).

The present infiltration of CA-MRSA to a health care or hospital setting tend to pose several clinical implications for effective disease control (Kennedy and Deleo, 2009). In the United States of America, ST8/*SCCmec* IVa also known as MRSA USA300, is one of the commonly characterized CA-MRSA clones presently recognized to cause hospital related infections (Sato *et al.,* 2017). The panton-valentine leucocidin (pvl) produced by CA-MRSA harbors *SCCmec* type IV or V and causes massive tissue necrosis plus the destruction of leukocytes (Sato *et al*., 2017).

2.4.1.3 Livestock associated MRSA (LA-MRSA)

Since its initial recognition in the Belgium in 1972 as an extensive reservoir in pigs and cattle, poultry and horses, MRSA clonal lineages isolated from livestock has increasingly emerged as a problematic infectious agent in veterinary field and there after designated as Livestock Associated MRSA (LA-MRSA) (Chon *et al*., 2017).

In sub-Saharan Africa, commonly among the pastoralist communities who constantly interact with animals, stands at high risk of been colonized by *S.aureus* and its variant MRSA resulting in manifestations of serious infections in health care units, in the general population, livestock and livestock production systems (Omwenga *et al*., 2019).

To a large extent, LA-MRSA is linked to food of animal origin, but can equally colonize intra-species resulting to diseases in humans with close proximity with MRSA pre-colonized

animals such as pigs or calves (Graveland *et al*., 2011; Boswihi and Udo, 2018) even though 20% to 40% of ST398 strain of human MRSA cannot directly be associated to close companion animals therefore pointing out of possible existence of alternative transmission pathway (Sato *et al*., 2017). Reported LA-MRSA cases in European countries belong to ST398 and ST9 in Asian countries (Cuny *et al*., 2015; Sato *et al*., 2017), although in Italy other clonal complex such as CC1 and CC97 have also been reported to cause diseases in livestock (Locatelli *et al*., 2017; Giacinti *et al*., 2017).

2.5 MRSA between animals and humans

The growing prevalence of MRSA in some occupations and vulnerable groups is worrying more especially in the light of evidence that MRSA spreads freely between animals and humans (Klevens *et al*., 2006). A case study of the sequence type 398 (ST 398) which has been pointed out to be a colonizer and an infectious agent amongst the interspecies and intraspecies pose feasible threat (Cuny *et al*., 2010; Lozano *et al*., 2016).

Until recently, MRSA strain has been susceptible to a range of different classes of antibiotics other than βeta-lactams, however cross-resistance and multidrug resistance is on the rise (Boucher *et al*., 2010; Dadashi *et al*., 2018). Since 1990's the increase in epidemiology of MRSA infections arising from the community has been undertaken in many parts of the world and of late MRSA being pointed to colonize or infect both animals and humans (Köck *et al*., 2010).

MRSA strains can disseminate between people of close proximity with animals and this is held to be possibly true as recent studies shows DNA similarity at the molecular level between

MRSA isolates from animals and their by-products and humans. (Duquette and Nuttal, 2004)

The MRSA infectious variants in livestock have been identified amongst lineage of farmers more particularly CC398, which has been recognized in humans with no history of close proximity to animals and these strains tend to show multi-resistance characteristics (Lozano *et al*., 2016; Back *et al*., 2020). The closeness between animal and humans provides a higher opportunity for transmission between the multispecies group and after acquisition, further interspecies and intraspecies transmission of the resistant strain may occur (Morgan, 2008).

S.aureus infection to man is considered majorly to be transmitted through milk and its derivatives whereby in Europe its approximated to contribute up to about 5 % of all staphylococcal outbreaks (Jahan *et al*., 2015).

MRSA is no longer limited to humans as previously thought, but currently is a significant zoonotic pathogen (Locatelli *et al*., 2017). From the year 2006, the detection of MRSA of animal species such as dairy cattle, horses, pigs, chickens and turkeys, has demonstrated that largely livestock serves as harbours of MRSA belonging to clonal complex (CC398) which is zoonotic in nature (Sharma *et al*., 2016).

In The Netherlands, the surfacing of LA-MRSA marked concurrent increase of MRSA cases in humans between the year 2001 and 2006 (Graveland *et al*., 2011). The percentage proportion of long-period harbour of MRSA for a period exceeding one year (>than 1 year) falls between 10% and 20 % (Graveland *et al.,* 2011).

In the recent past, another clone of MRSA known as *mecC* formerly called *mec*A$_{LGA251}$ (Petersen *et al*., 2013) which share molecular similarity up to 70 % with *mec*A (Ballhausen *et al*., 2014) has been detected both in humans and animals, with isolates of CC130, CC425 and CC1943 being reported from various European countries (Deplano *et al*., 2014**),** although the zoonotic transmission of *mec*A$_{LGA251}$ previously reported, data is still scanty on the prevalence, epidemiology and animal reservoirs (Giacinti *et al.,* 2017).

Not only does mastitis compromise the quantity and quality of milk in dairy industry, but it's equally associated with significant zoonotic potential attributed to wide dissemination of the bacterial toxins through milk and its byproducts (Abebe *et al*., 2016).

The dissemination of *S. aureus* and its variant MRSA between interspecies has more often been reported in many developed countries more especially in regions of high livestock farming, pointing out to LA-MRSA (CC398) and CA-MRSA clonal complex CC97 with ease interspecies transmission (Schaumburg *et al*., 2015).

2.6 Global distribution of MRSA

The prevalence of MRSA worldwide including Africa in relatively one decade ago, as reported in various articles seems to be higher in specific regions than it was before the year 2000 (Falagas *et al*., 2013). In the Asian countries, MRSA prevalence of between 70 % and 80 % among *S. aureus* isolated from hospitas were reported (Song *et al*., 2011). In the European Union, MRSA prevalence ranges from <1% in countries such as Iceland, Netherlands, and Belgium to > 50% in Romani and Portugal (Falagas *et al*., 2013). Reports from Mediterranean

European countries such as Greece, Cyprus and Italy, MRSA prevalence rate ranges from 25% to 50 %, but extends to 56 % in Israel and 59 % in Malta (Falagas *et al*., 2013).

African countries neighbouring the Mediterranean sea, reported heterogenous cases of MRSA from one country to another for example in Libya, MRSA prevalence is reported to be 31%, in Algeria and Tunisia the prevalence rate is 45 % and 52 % in Egypt with Morocco reported to have the lowest MRSA prevalence rate of 19 % (Falagas *et al*., 2013).

Knowing and comprehending epidemiological data on MRSA in the continent is a crucial make-up of mind because documented review indicates that from the year 2000, the continuous uptrend of MRSA cases has become aglobally concern (Falagas *et al.,* 2013; Dadashi *et al*., 2018). Published studies on MRSA incidences, in the last few decades indicate an extensive rise of infections caused by *S. aureus* and its variant MRSA strain the worldwide (Dadashi *et al*., 2018).

The dispersion of MRSA strains between community and hospital setting has rendered the dichotomous ranking difficult (Deurenberg and Stobberingh, 2009). It's postulated that about two billion people globally harbours S. aureus and out of these, 53 million people are MRSA carriers (Rahman *et al*., 2018)

2.6.1 Epidemiology of MRSA in Europe

In the United Kingdom, cases of MRSA were first reported in 1961 (Enright *et al*., 2002; Oliveira and de Lencastre, 2002) exactly two years after methicillin were introduced into clinical practice (Enright *et al*., 2002).

According to Dulon *et al.,* (2011), there has been considerable increase in MRSA strains of *S. aureus* isolates between the years 1999 to 2002 in European countries more especially Netherlands, Germany, United Kingdom, Belgium and Ireland with MRSA prevalence ranging from as low as < 1 % in the northern Europe to 40 % in western and southern Europe (**Fig.2.3**). Recently published articles points out that in Europe, MRSA carrier rate is 4.6 % among healthcare workers of which 5.1 % show clinical symptoms of MRSA infection (Dulon *et al*., 2011).

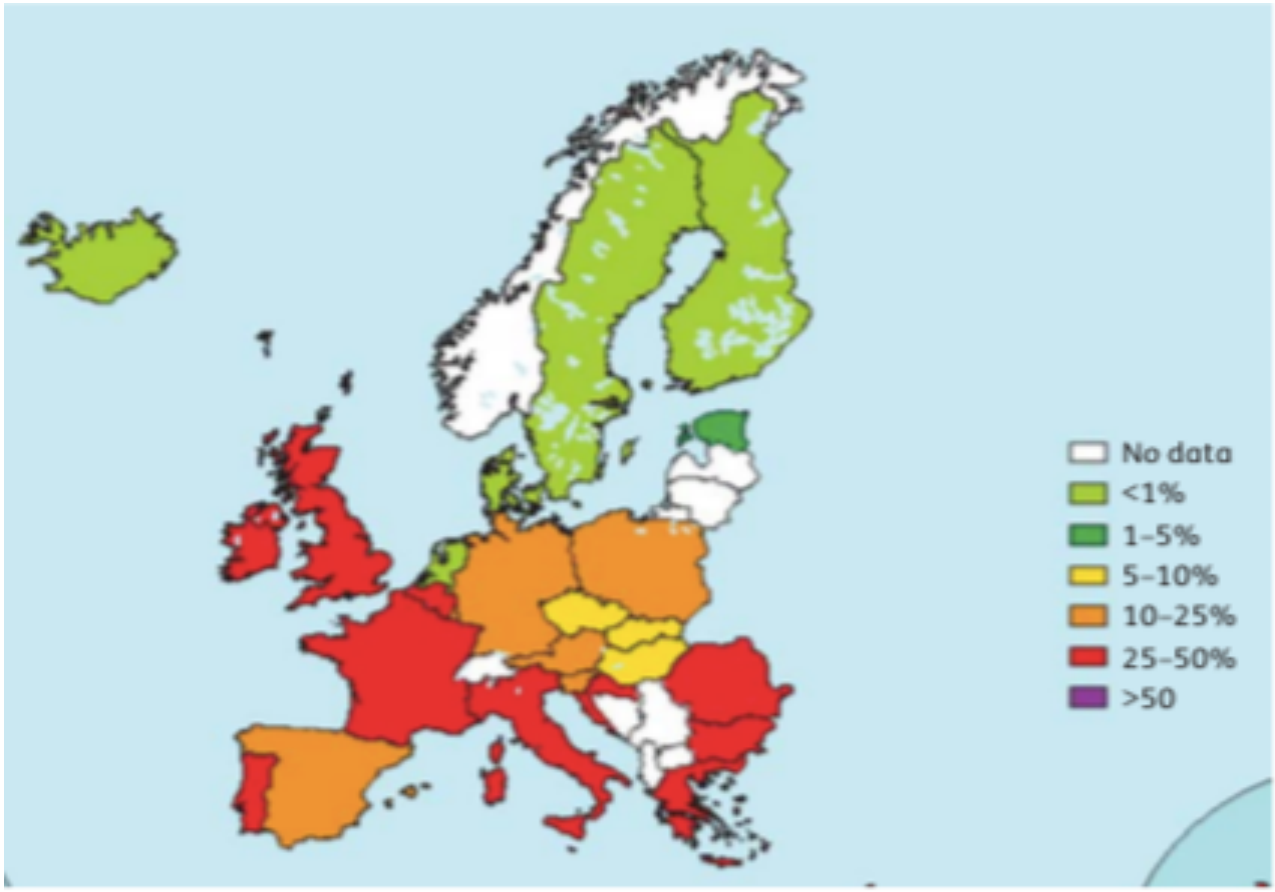


Fig. 2.3 Quotient of *S. aureus* isolates from blood resistant to methicillin in countries participating in EARSS, 2002 (EARSS Annual Reports).
Source: Johnson, (2011).

In the UK there has been dramatic day after day increase in proportion of *S. aureus* as a result of bacteremia that are methicillin resistant, reflecting the wide spread of two new MRSA strains designated as EMRSA-15 and EMRSA-16, and of these, EMRSA strain has shown to

be commonly resistant to ciprofloxacin, erythromycin and β-lactam antibiotics (Johnson, 2011).

2.6.2 Epidemiology of MRSA in United States of America

In the United States of America, ST8/*SCCmec* IVa also known MRSA USA300 is one of the commonly characterized CA-MRSA clones presently recognized to cause hospital related infections (Sato *et al* 2017). The first MRSA case in the USA was documented in 1968 (Raygada and Levine, 2009).

Available data from Denmark, Norway, Sweden, Austria and Canada demonstrates a rise in MRSA in blood majorly due to community associated infections therefore signifying that community based MRSA related diseases still remains a clinical challenge (Hassoun *et al*., 2017). In the year 2005, MRSA infections of invasive nature occurred in US at a rate of 31,800 (31.8 %) per 100,000 people and 75 % of these MRSA invasive infection were *S. aureus* bacteremia (SAB) that causes metastatic infections (Hassoun *et al*., 2017). Between 2005 and 2012, MRSA bloodstream infections declined yearly by 17.1 % however didnt greatly deviate in the years 2013 to 2016 (P=0.25); on the other hand, between 2005 and 2016, incidences of community acquired MRSA bloodstream infection rates decreased by 6.9 % (P<0.001) (Kourtis *et al*., 2019).

A comparative data analysis generated from 447 hospitals on hospital acquired and community acquired MRSA and MSSA and their associated mortality between the years 2012 to 2017, indicates 7.3 % (P <0.0001) decline of hospital acquired MRSA bloodstream infections per year, without great change in community acquired MRSA (Kourtis *et al*., 2019). Another study

carried out in 2010 on MRSA distribution among the American states reveals that, out of 67,412 hospital admitted patients, 4,476 patients are due to MRSA related infections with prevalence of MRSA standing at 66.4 % in every 1,000 hospital admitted patients (Jarvis *et al*., 2012).

2.6.3 Epidemiology of MRSA in Asia

In Asian countries, the dissemination of MRSA between health care setting and community setting has increased tremendously that the prevalence in Hong Kong, Singapore, Japan, and Taiwan, is greater than 60 % (Diekema *et al*., 2001), and equally high rates of methicillin resistance have been documented in previous articles (Ho *et al*., 1999). MRSA accounts for 25.5 % of all community setting *S. aureus* related diseases while 67.4 % covers healthcare setting infections ((Song *et al*., 2011). The Asian-Pacific region is one of the highly populated regions in the world with high rate of urbanization and a reasonable number of people live in high-density cities, these factors enhance the risks of antimicrobial resistance (Lim *et al.,* 2019).

Since the detection of MRSA in 1980 in healthcare setting within the Asian regions, the prevalence proportions has tremendously increased ranging from 26 % to 73 % in 2011 (Lim *et al.,* 2019). Even though HA-MRSA cases have decreased in Taiwan and Japan from the year 2000, the CA-MRSA infections are still high on the region (Lim *et al.,* 2019).

In Taiwan, MRSA among all the nosocomial *S. aureus* raised from 20.2 % in 1981 to 69.3 % in 1999 with an overall MRSA prevalence rate of 60 % for all isolates of *S. aureus* and 50 % for bloodstream from the year 1998 to 2000 (Chen and Huang, 2014). In Japan, a study encompassing 43 hospitals in 1990 shows MRSA prevalence rate of 58.6 % for all *S. aureus*

clinical isolates and the rate raised from 67 % in 1998 to 71.6 % in 2001 (Chen and Huang, 2014).

In Korea, Nationwide surveillance in 1998 for all clinical *S. aureus* isolates shows MRSA prevalence rate of 72 % from 25 hospitals and generally HA-MRSA remains extremely high (Chen and Huang, 2014). In Hong Kong and China, the MRSA cases remained relatively low in early 2000s at prevalence of 13 % to 27.8% but between 2004 and 2005, the prevalence increased to 50-62 % (Chen and Huang, 2014).

Study done by ANSORP between 2004 and 2006 in Southeast Asia, shows MRSA prevalence rate of 74.1 % in Vietnam, 57 % in Thailand and 38.1 % in 38.1 % and another multinational study of RSS program carried out in 2011, shows MRSA prevalence among clinical isolates of *S. aureus* falling between 28 % in Indonesia and 59 % in Philippines (Chen and Huang, 2014). In South Asia, ANSOPR report between 2004 and 2006 show MRSA prevalence rate of 86.5 % in Sri Lanka (Chen and Huang, 2014) and MRSA rate of 41 % and 45 % in the year 2008-2009 and 2011 respectively while in Pakistan, multicenter study between 2006 and 2008 showed prevalence rate of 41.9 % similar to one reported in Sri Lanka (Chen and Huang, 2014).

2.6.4 Epidemiology of MRSA in Africa

The MRSA prevalence and distribution in most African countries is heterogeneously updated and not accurately described due to poor antimicrobial resistance monitoring systems and and stewardship, accompanied with limited data collectetion across the continent (Falagas *et al*., 2013; WHO, 2014; Garoy *et al.,* 2019).

African healthcare system as a whole extremely suffers from inadequate trained healthcare personnel, poor infrastructure, shortage of medical equipment and inappropriate medications and financial crisis which in overall enhances the spread and increased trends of communicable and non-communicable diseases consequently overburdens the few functional healthcare system ((Falagas *et al*., 2013).

In Africa, MRSA cases were reported first in 1978 but hospital outbreaks were experienced between 1986 and 1987 in South Africa, while CA-MRSA infections were reported in 1990s in Zimbabwe (Falagas *et al*., 2013). Mediterranean Region, reported MRSA with median prevalence of 39 % but slightly higher (>50 %) in hospitals from Malta, Cyprus, Egypt and Jordan (Borg *et al*., 2010)

Published studies show heterogeneous MRSA prevalence rates across country and within country for example in Cameroon, prevalence of 47 % (95 % CI), Ethiopia, prevalence of 33 % to 61 %, in Uganda, 31.5%, in Eritrea 9 % and Kenya with prevalence of 3.7 % (Garoy *et al*., 2019). Documentary data on nine African countries approximates MRSA prevalence of 12 % on the lower and 80 % on the upper but some countries have reported MRSA prevalence rate exceeding 82 % (Wangai *et al*., 2019). In Cameroon, among the 295 samples that were analysed on antibiotic resistance profile in hospitalised adult patients and medical staff, 40.6% isolates were confirmed positives for *S. aureus* out of which 34.6 % were MRSA (Gonsu *et al*., 2013).

In East Africa countries like Rwanda, Tanzania and Uganda, MRSA prevalence is heterogeneously distributed between 31.5 % and 42 % Uganda, 31 % to 82 % in Rwanda and

10 % to 50 % in Tanzania (Wangai *et al*., 2019) but in South Africa MRSA prevalence rates declined from 34 % to 28 % in 2011 (Wangai *et al.,* 2019). High rates of MRSA were observed in Kenya, Cameroon and Nigeria ranging between 21 % and 30 % however Algeria, Malta and Tunisia recorded less 10 % (Kesah *et al*., 2003).

2.6.5 Epidemiology of MRSA in Kenya

MRSA prevalence in Kenya has been inconsistent due to inadequate and ineffective surveillance systems to monitor trends of antimicrobial resistance (Wangai *et al*., 2019) and more commonly Kenya data on MRSA is poorly reported or not recorded at all (WHO, 2014) but a study carried out on the proportions of MRSA from skin and soft tissue infection among the inpatients in Nairobi indicated a prevalence of MRSA of 84.1 % (Maina *et al*., 2013).

MRSA prevalence rate of 53.4 % was reported from amongst a sizable number of *S. aureus* isolates obtained from adult patients in the general medical ward and 50.6% from paediatric surgical ward at Kenyatta National Hospital (Wangai *et al*., 2019). Another cross-sectional study carried out in two non-governmental health facilities in Nairobi, shows 3.7 % MRSA prevalence for general specimen types and 6.5 % from blood isolates (Omuse *et al*., 2014) while 6.9 % was noted at one government hospital in Thika (Aiken *et al*., 2014) and Njoroge (2016), reported prevalence of 7% among dogs.

2.7 Virulence mechanisms for MRSA

The virulence mechanisms of *S. aureus* and its variant MRSA strains is multi- functional and dependent on production of toxins, adhesion proteins, adaptation of gene expression, ability to colonize through mobile genetic elements and immune evasion mechanisms (Otto, 2012).

S. aureus toxins and its associated virulence mechanisms are coded on mobile genetic elements (MGEs) (Malachowa and DeLeo, 2010) and this includes; MGE- encoded toxins such as Superantigens like toxic shock syndrome toxin (TSST), leukotoxins like exfoliative toxins and Pvl (Watkins *et al*., 2012).

The surface proteins such as SasX proteins, which are encoded on MGEs, assist in MRSA colonization and promote aggression of the bacteria and consequentially resulting to decreased neutrophil phagocytosis (Otto, 2012).

Phenol-soluble modulins-mec (PSM-mec) gene, belonging to a class of *S. aureus* peptide proteins coded on MGEs exhibits cytolytic activity to erythrocytes and neutrophils to humans and animals (Otto, 2012; Watkins *et al*., 2012). Resistant chromosomal genetic elements present in *S. aureus* and its variant MRSA strains occurs through acquiring mecA gene or mecC, all situated within the large SCCmec chromosomal element (Lakhundi & Zhang, 2018).

Another large mobile genetic element known as Arginine catabolic mobile element (ACME) is of great clinical significance in pathogenesis and transmission of CA-MRSA (Watkins *et al*., 2012). Complicated MRSA related infections more especially among patients with prosthetic joints and catheters are due to the capability of MRSA to form biofilms (Watkins *et al*., 2012).

2.8 Laboratory methods of diagnosing MRSA strains

Laboratory methods for detection of MRSA in clinical samples encompass both conventional approaches and molecular methods (Brown *et al.,* 2005). The conventional method involves the use of standard solid culture media with or without pre broth enrichment as described by

Kirby-Bauer disc diffusion method for identification of methicillin resistant coagulase positive staphylococci and the interpretation of results is based on measuring diameter zone of inhibition of ≤ 21 mm for disk diffusion using *S. aureus* ATCC ® 25923 for disk diffusion as standards. Clinical specimens from suspected sources of infection for analysis include but not limited to nasal swabs, blood, sputum, urine, or wound scraping in humans and milk

Molecular method included the use polymerase chain reaction (PCR) primers for amplification of *mec*A and *mec*C gene using specific primers and then sequencing the *mec*A and *mec*C positive isolates however the method is only applicable for identification of pure isolates of *S.aureus* and cannot be applied directly on crude samples (Brown *et al.,* 2005).

2.9 Therapeutic management of MRSA infections

The choice of antibiotic therapy for management of MRSA related infections is purely guided by the type of disease in question, availability of the drug, *S. aureus* resistance pattern, individual patient factors and drug side adverse effect profile (Siddiqui and Koirala, 2020). Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) for the treatment of MRSA infections in adults and children recommends a wide range of antibiotics for curbing S.aureus infections in a clinical setup and these includes; βeta lactams (cephalexin, dicloxacillin, amoxicillin), Lincosamides (clindamycin, lincomycin), Sulfonamides (trimethoprim-sulfamethoxazole), Tetracyclines (doxycycline, minocycline) Oxazolidinones (linezolid), Glycopeptides (vancomycin, telavancin) and Lipopeptides (daptomycin) (Liu *et al*., 2011).

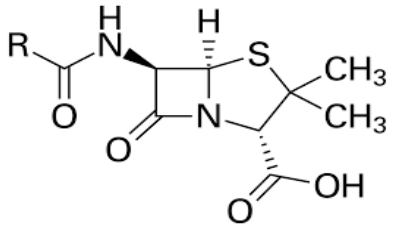
About 56 % of staphylococcal infections in rural healthcare cntres are caused by MRSA, while in some tertiary healthcare centres, MSSA is responsible for up to 75 % of all staphylococcal infections and empiric therapy with cephalosporins or penicillins offers appropriate clinical response (Loewen *et al.,* 2017). In the case of methicillin sensitive *S. aureus*, clindamycin, quinolone, trimethoprim-sulfamethoxazole (TMP-SMX), rifampin or doxycycline are recommended but due to emergence of resistance, the combination of trimethoprim-sulfamethoxazole (TMP-SMX) and rifampicin is recommended than singly (Lee *et al.,* 2011). Regions with low resistance rates to clindamycin, clindamycin rather than, trimethoprim-sulfamethoxazole (TMP-SMX) is recommended among the outpatient subjects (Williams *et al.,* 2011).

Strains of MRSA that produce β lactamase enzyme, oral or parenteral semi synthetic penicillins remains the preferred drugs of choice but for patients who are allergic to penicillins, first generation cephalosporins serves as alternative regimen (Bamberger and Boyd, 2005). For simple Skin and soft tissue infections (SSTIs) due to MRSA, empiric antibiotic approach with oral trimethoprim/sulfamethoxazole, clindamycin and tetracyclines, such as doxycycline or minocycline is first line drug of choice (Siddiqui and Koirala, 2020). In the management of MRSA related bacteremia, vancomycin and daptomycin is recommended but in cases where vancomycin or daptomycin is contraindicated secondary to toxicity, allergy or resistance, then telavancin and ceftaroline can be used as secondary available alternatives for bacteremia (Liu *et al*., 2 011; Siddiqui and Koirala, 2020).

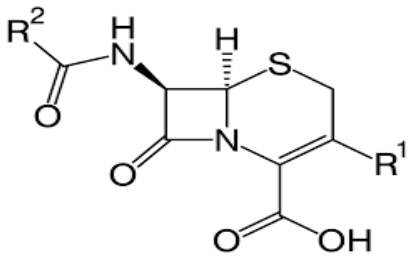
2.9 Classification of βeta lactam antibiotics

βeta lactam antibiotics are a class of antibiotics that are characterized by possession of β-lactam ring in their chemical ring structure and these includes carbapenems, oxapenems, cephamycins, penicillins, and cephalosporins (**Fig.2.4**). They are broadly classified and distinguished from one another based on the variation in the ring structure adjourning the β-lactam ring and the side chain at the alpha-position of β-lactams structure (Patrick, 2013).

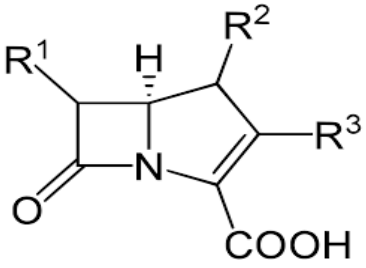
Penicillins



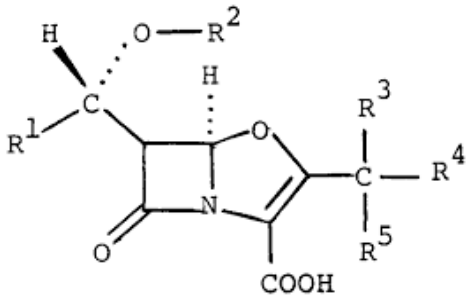
Cephalosporins



Carbapenems



Oxapenems



Cephamycins

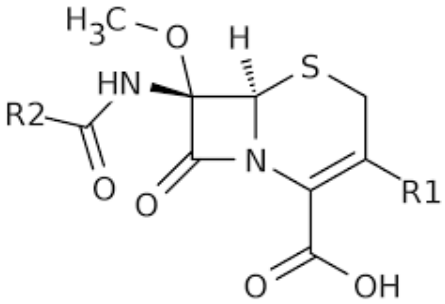


Fig. 2.4 Structures of some beta lactams antibiotics
Sourse: Patrick, 2013

βeta lactam antibiotics such as penicillins can further be sub-classified and distinguished from one another as either natural penicillin, penicillinase resistant penicillins, extended spectrum penicillins and aminopenicillins while cephalosporins are sub-classified based on thei generation (**Table 2.1**).

Table 2.1: Classification of β-lactam antibiotics, examples and spectrum of activity

Class	Sub-class	Examples	Spectrum of activity
Penicillins	Natural penicillins	Penicillin G, Penicillin V, Penicillin G. procaine, Penicillin G. Benzathine	Gram positive cocci and some G-ve cocci
	Penicillinase – Resistant Penicillins	Cloxacillin, Dicloxacillin, Methicillins, Naficillin& Oxacillin	Staphylococci e.g. *Staphy aureus, Klebsiella, Bacteroides fragilis*
	Extended spectrum penicillins (carboxypecicillin)	Mezlocillin, peperacillin, Piperacillin, Tazobactam, Ticarcillin, Ticarcillin / clavulanic	*Staphylococci ,klebsiella, Enterobacter Spp, Bacterioidfragilis, proteus, pseudomonas, Haemophilus Enterobacteriaceae*
	Aminopenicillins	Amoxicillin, Ampicillin Amoxillin/clavulanic acid Ampicillin/ Sulbactam	*Staphylococci , Klebsiella spp ,Bacteroides fragilis*
Carbapenems		Imipenem, Ertrapenem, Meropenem	Gram +ve cocci e.g. *Enterococci,*Gram –ve rods e.g. *pseudomonas aeruginosa, anaerobes, acinetobacter*
Monobactams		Aztreonam	G-ve rods including *pseudominas* and *serratia*
Cephalosporins	First generation	Cefadroxil, Cefazolin, Cephalexin, Cephapirin and cephradine	Gram +ve cocci except *Enterococci* Gram-ve bacilli e.g. *E. coli, Klebsiella pneumonia proteus mirabilis*
	Second generation	Cefaclor,Cefmetazole, Cefotetan,,Cefoxitin, Cefprozil,Loracarbef Cefuroxime	Gram+ve bacteria and extended Gram –ve bacteria including β-lactamases of *Haemophilus influenza*
	Third generation	Cefixime, Cefdinir,	Most Gram –ve bacteria

	Cefotaxime,Cefaperazone Cefpodoxime,,Ceftazidime Ceftibuten ,Ceftizoxime Ceftriaxone	e.g. *Enterobacter, ,Citrobacter, Serratia,,Providencia Neisseria* and *Haemophilus* organism including. β-lactamase producing strains
Fourth generation	Cefepime (Maximpime)	Gram+vecocci *Enterobacteriaceae,* and *pseudomonas*

Source: Petri, 2006.

2.10 Mechanisms of action of βeta-lactam antibiotics

βeta lactam antibiotics virtually act by interfering with enzymes-Penicillin Binding Protein (PBP) that are required in the synthesis of peptidoglycan layer (Džidić *et al.,* 2008). The PBP being covalently bound by the drug cannot perform the cross-linking activity and hence interfering with the bacterial cell wall synthesis. The inhibition of final step in bacterial cell wall peptidoglycan synthesis eventually results to bacterium cell death (Petri, 2006).

2.11 Mechanisms of antibiotic resistance

Microbial drug resistance is not a new phenomenon as microorganisms have constantly employed it as a survival tactic against the injurious effect of antibiotics. Presently antimicrobial resistance causing bacterium remains global threat to infectious disease management. To fight the offensive nature of antimicrobials, the microorganisms have evolved diverse mechanisms so as to avert the competitive challenges and destructive nature of antibiotics (Tanwar *et al*., 2014).

2.11.1 Resistance mechanisms to βeta-lactam antibiotics

The β-lactam ring in all β-lactam antibiotics (penicillin, carbapenem and cephalosporin) confers a very important activity in inhibiting a set of transpeptidases that play a prudent role in the last cross-linking step of bacterial peptidoglycan synthesis (Eyler and Shvets, 2019). The clinical effectiveness of β-lactam antibiotics relies purely on the ability of the drug molecule to reach and bind to penicillin binding protein (PBP) (Džidić *et al*., 2008). Various resistance mechanisms have been adopted by the susceptible micro-organism that enables them to respond to selective pressure of β-lactam antimicrobial agents and these includes;

(1). Target site modifications. The microorganism may intrinsically be resistant due to structural differences in PBP, which are the main target points for β-lactam antibiotics and because the affinity to receptor sites for β-lactam antibiotics decrease, the organism turns out to be resistant (Džidić *et al*., 2008; MacDougall and Chambers, 2011).

(2). Inactivation of antimicrobial agent. β-Lactamases, which are part of a group of classical hydrolytic amidases, may break-up the β-lactam ring, opening up the ring and therefore rendering the drug devoid of pharmacological activity (Petri, 2006).

Destruction of the drug molecule by the hydrolytic action of βeta lactamases, breaking up the amide bond of the lactam ring, consequently, rendering the antibiotic ineffective (Tenover, 2006; Jones *et al*, 2006; Munita and Arias, 2016). β-Lactamases constitutes diverse heterogenic enzymes, which are clustered differently based on their molecular localization, hydrolytic activity and gene or amino acid/protein sequences (Bush and Jacoby, 2010).

According to Bush and Jacoby (2010), functional classification scheme of β-lactamases is based on their capability to inactivate β- lactamase inhibitors such as

clavulanic acid, tazobactam and sulbactam or their ability to hydrolyse β- lactam class of antibiotics, βeta lactamases are classified into groups and the four categories includes:

Group 1 β-lactamase enzymes encompasses cephalosporinases, and they belong to molecular class C and are more active to cephalosporin than penicillin and are not well inhibited by clavulanic acid (Bush and Jacoby, 2010)

Group 2 β- lactamase enzymes, includes penicillinases, cephalosporinases and broad spectrum β- lactamases, belong to class A and D. They are commonly in Gram-positive cocci and tend to hydrolyse benzylpenicllin and their derivatives predominantly but relatively poor hydrolytic enzymes to eephalosporins, monobactams and Carbapenems (Bush and Jacoby, 2010).

Group 3 β- lactamases enzymes, are Metallo β- lactamases (MBLs) produced in combination with a second and third β- lactamases in clinical isolates. They are dependent of zinc ion at the active site. They have low affinity or hydrolytic ability for monobactam and are resistant to clavulanic acid or tazobactam (Bush and Jacoby, 2010)

Group 4 β- lactam enzymes, are penicillinases, which are resistant to clavulanic acid and also possess the functional ability to hydrolyse oxacillins (Bush and Jacoby, 2010)

(3) Changes or alterations of bacterium cellular permeability. Decreased antibiotic accumulation in the bacterium cell may consequently result to compromised critical concentrations of the drug that is required to exercise antibacterial activity, eventually leading to β- lactam antibiotics resistance (Cesur and Demiroz, 2013). The inability of antibacterial agent to penetrate through the peptidoglycan polymer is another myriad way through which bacterial can acquire resistance (Jacoby and Munoz-Price, 2005).

(4) **Mutations of the target site.** Alteration of penicillin binding proteins (PBP) results to low affinity to β-lactams and this may be acquired by homologous recombination between penicillin binding protein genes of different bacterial species (Džidić *et al*., 2008).

Some bacterium may develop resistance via mutation and genetic exchange at the receptor level such as acquisition of genetic element like *mec*A gene or *mec*C gene which is responsible for coding a modified penicillin-binding protein known as penicillin-binding protein-2a (PBP2a) which is the target receptor for β-lactam antibiotics resulting to decreased affinity to beta-lactam antibiotics including carbapenems with the exception ceftaroline and ceftobiprole (David *et al*., 2010; Boswihi and Udo, 2018).

2.11.2 Tetracycline resistance

Tetracyclines are most widely used antimicrobials in veterinary and human medicine. They act by blockin the attachment of amino acyl-tRNA group to the ribosomal receptor site, consequently this inhibits bacterial protein synthesis, and eventually death occurs (Chopra and Roberts, 2001; Rizvi, 2018).

According to Chopra and Roberts (2001), resistance to tetracyclines encompasses mainly three mechanisms, namely

Efflux pumps mechanism. Export of the drug from the bacterial cell occurs through an efflux protein (*tet* proteins) that belongs to major facilitator superfamily (MFS). All membrane-associated proteins that are coded with *tet* efflux genes export tetracycline from the bacterial cell thus reducing intracellular drug concentration, and this phenomenon tend to protect the intracellular ribosomes. Efflux proteins mostly confer

resistance against tetracycline with the exception of minocycline or glycylcylines (Chopra and Roberts, 2001).

Ribosome protection proteins. The action of tetracycline against the bacterium ribosomes is counteracted cytoplasmic proteins that enhance their protection ribosomes and this confers resistance to tetracyclines (Chopra and Roberts, 2001). The cytoplasmic proteins attach to the ribosome causing an alteration to the ribosome conformation and this action blocks tetracycline from attaching to the ribosome and therefore no stoppage of protein synthesis (Taylor and Chau, 1996).

Enzymatic inactivation of tetracycline. Some enzymes which are coded with resistance gene such as *tet* (X) gene promote enzymatic modification or alteration of tetracycline structure rendering the compound devoid of pharmacological activity and finally resistance to tetracyclines develops (Chopra and Roberts, 2001).

2.11.3 Chloramphenicol resistance

The action of chloramphenicol is dependent on the ability of this drug to bind to 50s ribosomal sub units reversibly consequently inhibiting peptidyl transferase enzyme activity and this blocks the binding of aminiacyl-tRNA to the receptor site in the final step in protein synthesis (MacDougall and Chambers, 2011). Chloramphenicol resistance occurs due to inactivation of the drug compound by chloramphenicol acetyl-transferase enzyme, which is produced by the control plasmid enzyme activity (Cesur and Demiroz, 2013; Egorov *et al*., 2018)

2.11.4 Aminoglycoside antibiotics resistance

Aminoglycosides achieves the desired pharmacological action by basically binding to polysomes (30s and 50s) ribosomal subunits and disrupts the normal processes in protein synthesis by causing misreading of template by incorporating erroneous amino acids into the

elongating polypeptide chain leading to premature termination of mRNA translation (MacDougall and Chambers, 2011; Egorov *et al*., 2018).

Mechanisms of resistance to aminoglycoside antibiotics are diverse and these includes; mutations of the ribosome target, ribosomal modification by ribosomal methyltransferase enzymes and inactivation of aminoglycoside by a family of enzymes referred to as aminoglycoside-methyltransferase enzymes (Garneau-Tsodikova and Labby, 2016).

Aminoglycoside modification and inactivation by a family of enzymes known as aminoglycoside-methyltransferase enzymes is the most single dominant mechanism underlying bacterial resistance to aminoglycoside antibiotics (Garneau-Tsodikova and Labby, 2016).

A good proportion of clinically important bacterial species possess numerous mechanisms ranging from a single plasmid possessing multiple resistant genes and they act synergistically to another mechanism in conferring antibacterial resistance, consequently these has resulted to increased aminoglycoside resistant phenotypes (Garneau-Tsodikova and Labby, 2016). Depending on the type of chemical modification these aminoglycoside-modifying enzymes brings about on the aminoglycoside structure; they are classified into three subclasses namely; Aminoglycoside- *O*-nucleotidyltransferases (ANTs), Aminoglycoside- *N*-acetyltransferases (AACs) and Aminoglycoside *O*-phosphotransferases (APHs) (Egorov *et al*., 2018; Garneau-Tsodikova and Labby, 2016).

Modifications of the aminoglycoside structure at amino group by Aminoglycoside- *N*-acetyltransferases (AACs) enzymes or at the hydroxyl group by the Aminoglycoside- *O*-

nucleotidyltransferases (ANTs or Aminoglycoside *O*-phosphotransferases (APHs) enzymes makes the aminoglycoside antibiotic to lose their ribosomal binding capacity, consequently the antibiotic becomes devoid in inhibiting bacterial protein synthesis (Quintiliani and Courvalin, 1995). Efflux pumps besides rRNA mutations and cell membrane modifications play an important function in reducing the bacterial intracellular concentrations of aminoglycosides and therefore serving as an additive mechanism in development of aminoglycoside resistance (Garneau-Tsodikova and Labby, 2016; Egorov *et al*., 2018).

2.11.5. Macrolides, Lincosamides and Streptogramin (MLS) resistance

The MLS class of antibiotics is chemically different from one another but possess similar mechanisms of action by inhibiting bacterial protein synthesis (Leclercq, 2002). Bacteria develop resistance to macrolides and lincosamides mainly through three mechanisms namely:

(1) Modification of the target site by methylation or mutation prohibits the antimicrobial agent from binding to the ribosomal target site. Post-transcriptional modification of 23SrRNA by methylase enzymes such as adenine-N6- methyltransferase are encoded by genes *erm* (erythromycin ribosome methylation) which modifies or change a common binding portion in 23S rRNA for all MLS_B antibiotics (Johnston *et al.,* 1998; Leclercq, 2002; Cesur and Demiröz, 2013).

(2) Efflux pump systems such as ABC transporters encoded by plasmid born msr (A) gene exports the drug from bacterial intracellular membranes and this maintains the intracellular drug concentration extremely low making bacterial ribosomes to be free of antibiotic and antibiotic action (Johnston *et al.,* 1998; Leclercq, 2002; Cesur and Demiröz, 2013).

(3) Antibiotic enzymatic inactivation. This tends to confer a wider spectrum of resistance to structurally related antibiotics (Johnston *et al.,* 1998; Leclercq, 2002; Cesur & Demiröz,

2013). Phosphotransferases encoded by *mph*(C) gene and esterases confer resistance to erythromycin with the exception of lincosamides but in *staphylococci* and *Enterococcus faecium,* lincosamide nucleotidyltransferases encoded by *lnu*(C) and *lnu*(B) inactivates lincosamides antibiotics only (Leclercq, 2002).

Hydrolytic enzymes confer resistance to streptogramins and structurally related antibiotics by hydrolyzing streptomycin-B or modifying antibiotic structure through the action of acetyltransferases making the drug devoid of bacterial protein synthesis inhibitory activity (Johnston *et al.,* 1998).

2.11.6 Quinolone Resistance.

The quinolones class of antibiotics acts by formation of DNA-typeII topoisomerase complex between the DNA and topoisomerase II or IV consequently blocking replication and transcription of bacterial DNA eventually resulting to bacterial death (Hawkey, 2003; Nordmann and Poirel, 2005).

DNA gyrase consists of two subunits designated as A and B and are designated as *gyrA* and *gyrB* genes and this enzyme is responsible for unwinding the topological tension due to translocation of transcription and replication complexes along DNA besides introduction of negative supercoil into the DNA (Hawkey, 2003; Nordmann and Poirel, 2005; Jacoby, 2005).

Topoisomerase IV, which is a homologue of DNA gyrase, is basically involved in separation and unlinking of replicated daughter chromosome (Nordmann and Poirel, 2005) and is known

to comprise four subunits, two Cs and two Es and they equally harbour *parC* (also referred to as *grlA* in *S. aureus*) and *parE* genes respectively (Hawkey, 2003; Jacoby, 2005).

The two enzymes (DNA gyrase and DNA topoisomerase IV) work in a complementary manner in the replication, transcription, recombination, and repair of DNA and the whole process is an ATP dependent reaction (Jacoby, 2005). Resistance in quinolones to clinical pathogens has been a problem for more than four decades since the introduction of nalidixic acid (Jacoby, 2005)

Mechanisms of bacterial resistance to quinolones occur in three ways namely; (1) Drug target enzyme alteration or mutations. (2) Plasmid protection from the destructive effect of the quinolones. (3) Mutations that limit drug permeability to the target site (Hooper, 1999; Jacoby, 2005; Nordmann and Poirel, 2005).

2.11.7 Sulfonamides and Trimethoprim Resistance

Sulfonamide act by competitively inhibiting the bacterial enzyme dihydropteroate synthase (DHPS), which catalyze the final steps, and the condensation of p-aminobenzoic acid to dihydropteroic acid, in a stepwise cascade of reaction leading to the synthesis of dihydrofolic acid (Huovinen, 1995; Ola, 2001 and 2010).

Trimethoprim act by competitively inhibiting the reduction of dihydrofolate to tetrahydrofolate by an enzyme dihydrofolate reductase (DHFR), an enzyme catalyzed reaction leading to the synthesis of bacterial DNA thymine. The human dihydrofolate reductase enzyme is endogenously resistant to trimethoprim, which is the basis for its selectivity and its clinical use (Huovinen, 1995; Ola, 2010).

Trimethoprim resistance is due to mutational change in intrinsic *dfr* gene that encodes a resistant dihydrofolate reductase enzyme and mutations on the structural gene for dihydrofolate reductase enzyme and also over expression of dihydrofolate reductase enyme by the host (Thomson, 1993; Ola, 2001).

2.11.8 Multidrug Resistance (MDR) *S. aureus*

Multidrug resistance is a phenomenal non-susceptibility of an isolated microorganism to at least one antimicrobial agent in three or more antimicrobial categories (Tanwar *et al*., 2014). Multidrug resistance to many microorganism has posed a feasible clinical challenge to infectious disease management with an overall enhanced morbidity and mortality therefore referred to as "super bugs" (Tanwar *et al*., 2014).

Multidrug resistance in bacteria is commonly brought about by the accumulation genetic mobile elements such as integrons, transposons, and plasmids or through resistant genes for specific antimicrobial agent and lastly by the action of multidrug efflux pumps (Nikaido, 2009). Transposons are discrete DNA segments with mobile ability from one genetic location to another and within them they carry genes encoding transposition proteins and DNA sites at the element termini that participate directly in transposition (Arciszewska *et al*., 1989: Muñoz-López and García-Pérez, 2010). Integrons are mobile DNA segments that possess a site-specific recombination mechanism capable of integrating, expressing and interchanging gene cassettes (Gillings, 2014) and they play a significant role in promotion and spread of multidrug resistance gene across gram positive and gram-negative bacteria (Deng *et al*., 2015). Type I integrons, are the most common and encodes sulfonamide resistance gene *sul1* while class II integrons, are associated with Tn7 and carries resistance to streptomycin and trimethoprim

(Arciszewska *et al*., 1989). Majority of these mobile genetic elements promotes the spread and expression of multidrug resistance to a wide category of antimicrobial agents including; penicillins, macrolides, chloramphenicol, cephalosporins, trimethoprim, tetracycline and aminoglycosides (Magiorakos *et al*., 2012).

2.12 Mechanism of MRSA resistance

MRSA strains shows a unique characteristics with regards to multi-drug resistance phenomenon across the βeta lactam and non-β-lactam antibiotics because of its ability to intergrate *Scc-mec* elements into the chromosome therefore converting susceptible *S. aureus* to be resistant to many antimicrobials (Hiramatsu *et al*., 2001; Okon *et al*., 2011).

The *mec*A geneis responsible for coding a modified penicillin-binding protein called penicillin-binding protein-2a (PBP2a) which is the target receptor for β-lactam antibiotics resulting to decreased affinity to beta-lactam antibiotics including Carbapenems with the exception of the ceftaroline and ceftobiprole (Okon *et al*., 2011; Abdulgader *et al*., 2015; Boswihi and Udo, 2018).

Evaluation of whole gene sequencing, mutations of the endogenous penicillin binding proteins 1, 2 and 3 in *mec*A (Ba *et al*., 2014) there is *mec*C gene which is a homolog of *mec*A, shares up to 70 % nucleotide similarity with *mec*A (Ballhausen *et al*., 2014), consequently MRSA isolates harbouring *mec*C from humans and animal have been postulated to be alternatively possible mechanisms for resistance of MRSA βeta lactam antibiotics (Abdulgader *et al*., 2015).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Reference Bacteria

S. aureus (ATCC 25923) purchased from Oxoid LTD, Basingstoke, Hampshire, (England) and maintained in the Department of Public Health, Pharmacology and Toxicology, College of Veterinary Sciences, University of Nairobi was used as the reference strain.

3.2 Chemicals, Glassware, Media and Plastic ware

Mannitol Salt Agar (MSA), Tryptone Soya Broth, Mueller-Hinton Agar, Skim Milk Powder, Blood Agar, Buffered Peptone Water all were obtained from Oxoid LTD, Basingstoke, Hampshire, England. Analytical grade chemicals for Molecular Biology were purchased from Oxoid LTD, Basingstoke, Hampshire, England. Glassware (conical flasks 500ml, 250ml, 100ml, graduated reagent bottles wide mouth screw cap, 15ml test tubes, petri plates) was procured from Borosil, India. Plastic ware (1.5ml, 2ml eppendorf tubes) (1-10µl, 20-100µl, 200-1000µl micro tips) of Tarsons products pvt ltd was used in this research.

3.3 Equipments

The equipments utilized in this study have been stated respectively wherever necessary and among the many includes Refrigerated System Centrifuge (Thermo, Germany), UVP CelMax 125 Imager (Upland, CA USA), Eppendof Centrifuge AG 22331 (Hamburg Germany), AB Applied Biosystems Veriti 96 well Thermal Cycler (USA) Laminar Air flow (Thermo, USA), Gel documentation System (Bio-Rad, USA), Nanodrop 200C (Thermo, USA) and horizontal electrophoresis (Atto, Japan).

3.4 Study Sites

3.4.1 Human blood sample

The study was carried out on clinical blood from human patients attending healthcare facilities at Mukuru slum, Nairobi County (**Fig. 3.1**). Mukuru is second-largest slum community in Kenya comprised of Viwandani, Mukuru Kwa Reuben and Mukuru Kwa Njenga, covers an area of 525 acres, comprising about 100,561 households, with population of 700,000 residents (Kenya National Bureau of Statistics, 2009). This slum is densely populated and is faced with myriad of social challenges including infrastructure for providing clean water, sanitation facilities, solid-waste management, roads, drainage, and electricity. The residents of this slum experience high incidences of diseases such as diarrhea, upper respiratory tract infections, malaria, fever and other communicable diseases (United Nations Commission on Human Settlements, 1996).

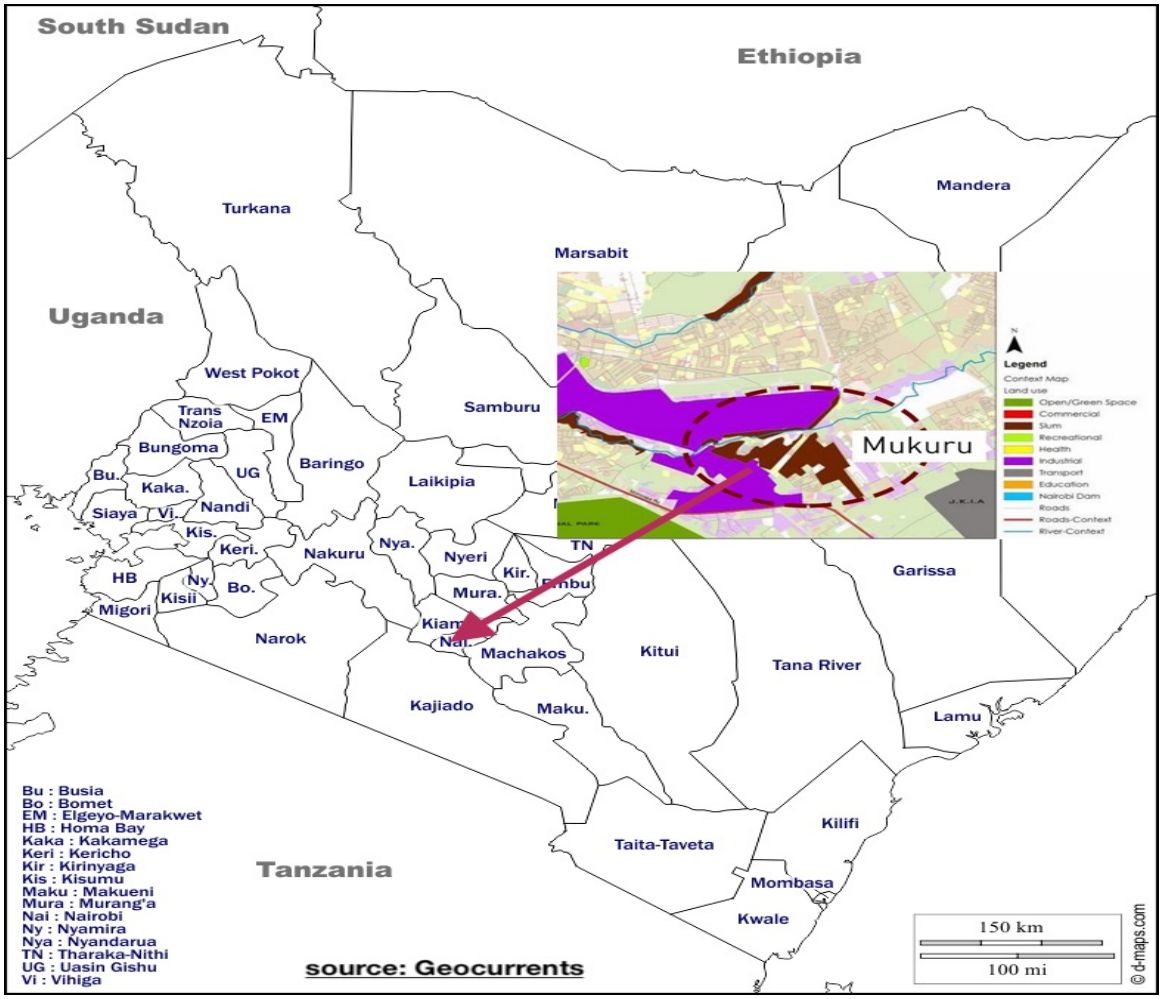


Fig. 3.1: Map showing Mukuru Kwa Njenga slums.

3.4.2 Dairy milk samples

Milk samples were collected from five selected livestock farms in Peri-urban Nairobi, (**Fig. 3.2**) between November 2016 and October 2017, in a one off sampling process from each animal teat. The five randomly selected dairy farms for milk sampling came from two different parts of Kiambu and Kajiado counties that neighbours Nairobi County where human samples were obtained. The specific dairy farms randomly selected from Kiambu county included: Kanyariri farm (n=103), Dominic farm (n=107), Kabogo farm (n=50) and Karuga farm (n=40) whereas from Kajiado county, milk samples were from Ngong veterinary farm (n=53).

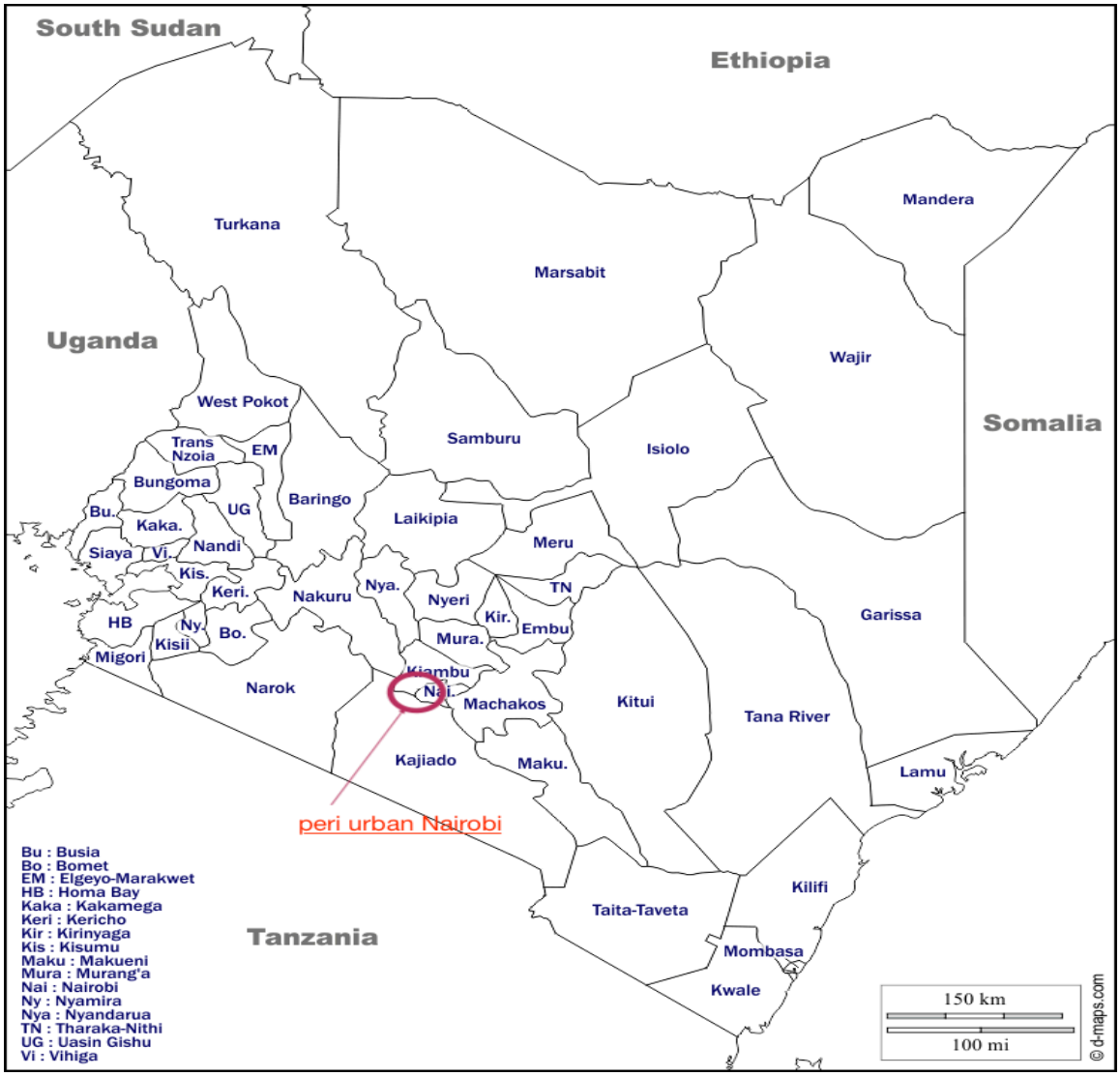


Fig. 3.2: Map showing Peri Urban Nairobi

In this study, the samples were independently obtained from different non-related locations to study the specific gene in circulation that is conferring resistance to methicillin and related β-lactam antibiotics at the molecular level for both humans and animal species.

3.5 Study Design

3.5.1 Human blood

The out patients were approached for informed consent for participation in this study using

inclusive and exclusive criteria. Inclusive criteria for a patient to participate in this study included: agreed consent, must be a resident in the Mukuru slum, presented with a subjective history of at least 3 days of fever and have an axillary temperature of at least 37.5 ^{o}C or they presented with a history of fever of any duration and have an axillary temperature of at least 37.5 ^{o}C; or they reported having had three or more loose or liquid stools (children > 2 years) or 8 or more for infants in the 24 hours before presentation, or one or more loose or liquid stool with visible blood. Exclusion criteria included absence of informed consent. Individual health facilities were attributed an identification code for anonymization. Because of unpredictability of the number of patients who could present with the desired clinical complaint, sample size calculation was not determined. A total of 142 blood samples were collected by KEMRI medical doctors with the assistance of other healthcare workers in a one ran off sampling process between November 2016 and October 2017. The blood samples collected were immediately labeled using unique reference numbers and then transported inside a cooler box with a warm pack to the Department of public health, pharmacology and toxicology, faculty of veterinary medicine, University of Nairobi, for bacteriological analysis

3.5.2 Milk sample

Milk samples were collected from farms in Kiambu and Kajiado, specifically those neighbouring Nairobi County, between November 2016 and October 2017,

Farms that had thirty or more dairy animals were considered. Ten farms were identified to having thirty dairy animals and above and then five dairy animals were randomly selected from the possible ten farms. The Inclusive criterion involved sampling from all lactating animals from the selected farms. Exclusion criteria included absence of informed consent. The commercial farms included in this study sell milk directly to local residents or regional travelers or to other commercial farms. The milking process in the commercial farm is either

mechanized or by hand. The milk samples were transported in room temperature to the Department of public health, pharmacology and toxicology, faculty of veterinary medicine, University of Nairobi, for culture and identification.

3.6 Sample Collection and Handling

3.6.1 Milk sample collection and handling

Physical examination of the udder quarters of each animal was performed for viability, and only those in the lactation phase and with at least one viable teat were included in this study, therefore, the milk sampling per animal ranged from one to four teats. None of the cows from in this study presented with any clinical symptoms related to mastitis or were under antibiotic therapy. The udder quarters with viability were cleaned with cotton soaked in water and soap, and then disinfected the udder and the teat with 70% alcohol. For each teat, the first three milk jets were discarded, and an aseptic milk sample (approximately 10 mL) was collected in a sterile tube (one tube per teat), conditioned at 2–8 ^{0}C in a cool box and transported to the Department of public health, pharmacology and toxicology, faculty of veterinary medicine, University of Nairobi, for culture and identification for culture and identification

3.6.2 Human blood sample collection and handling

The skin at the venepuncture site was sterilized using 0.5 % chlorhexidine in 70 % alcohol and allowed to dry before blood was withdrawn. Blood samples of 5mL from adults, 2mL for children and 1mL for neonates were aseptically obtained using venepuncture technique and drawn directly into sterile blood culture bottles containing sodium polyanethol sulfonate (SPS) as an anticoagulant and immediately the screw-cap was tightened by the attending clinician. The blood samples were then placed inside zip locks and placed into a cool box without ice

packs. The blood culture bottles were transported in room temperature to the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, for culture and identification.

3.7 Bacterial isolation and identification

3.7.1. Isolation of bacteria from milk samples

For isolation of *Staphylococcus spp*. from milk, 10 ml of the milk sample were inoculated into Buffered Peptone water and incubated at 37 ^{0}C for 18-24 hrs. After which, a loop full of inoculum from the culture tubes was streaked on Mannitol Salt Agar (MSA) while observing the aseptic procedures. The plates were incubated at 37 ^{0}C for 24hrs. *S. aureus* were ascertained based on colony morphology and pigment production viz; small, circular, smooth, golden-yellow colonies on Mannitol salt agar.

3.7.2. Isolation of bacteria from blood samples

For isolation of *Staphylococcus spp*. from blood samples, the specimens were sub cultured in Tryptic Soy Broth and then incubated at 35 ^{0}C to 37 ^{0}C for 3 – 7 days for signs of microbial growth which included any of the following:- floccular deposit on top of the blood layer or production of gas or white grains on the surface or deep in the blood layer. With the aid of a loop wire, the culture broth was streaked on to Mannitol salt agar (Oxoid) and then incubated aerobically at 37^{0}C for 22- 48 hours. Cultures showing characteristically bright to golden-yellow colonies were subjected to classical biochemical tests (Begum *et al*., 2011).

3.8 Biochemical Test for *S.aureus*

3.8.1 Slide Catalase test

Two drops of the hydrogen peroxide 3 % v/v solution were poured onto a microscopic slide. *Staphylococcus aureus* colonies from Mannitol Salt Agar were selected carefully using loop wire and then immensed in two drops of hydrogen peroxide solution on a glass slide to observe the catalase activity of the presumptive micro-organism. Active bubbling constitutes a positive confirmation of catalase positive test, and no bubble is a negative catalase test (Cheesbrough, 2006).

3.8.2 Tube coagulase test

Rabbit plasma (1ml) was pipetted into a test tube and then two pure colonies of *S.aureus* were added into the plasma. The contents were mixed gently and the test tube incubated at 37 ^{0}C. Clotting reaction was examined after one hour and then after three hours. If the test still remained negative, the test tubes were further stored at room temperature for 24 hours and the clotting phenomenon examined again by tilting each test tube gently. Any grade of coagulation, ranging from a loose clot suspended in plasma to a semi-solid clot, was conceived as positive result. *S. aureus* ATCC 25923 was used as positive control organism by subjecting the bacteria under the same conditions as the test organism (Enright *et al*., 2002).

3.9 Hemolytic activity

The haemolytic action of *S. aureus* isolates was done using 7% sheep blood agar. A 7 % sterile defibrinated sheep blood was added and gently mixed with earlier prepared blood agar and then 16 ml of the suspension was dispensed into petril dishes in a laminar flow and allowed to solidify. The media was streaked with *S.aureus* cultures and incubated at 37^{0}c for 24 hour and then the phenomenal β-haemolysis demonstrated by *S.aureus* examined. The observation of a

clear zone encompassing the colony is a positive confirmation of β- haemolysis while the greenish zone signifies of α- haemolysis (Forbes, 2007).

3.10 Preservation of *S. aureus* isolates

Five colonies of the *S. aureus* isolate were mixed with 1.5 ml of sterile skimmed milk contained in Cryovial tube and then tightened properly with a cock then stored in a refrigerator at temperature – 20 ^{0}C awaiting molecular characterization of nuclease gene and methicillin resistant gene *mec*A

3.11Antimicrobial susceptibility testing

Antimicrobial sensitivity testing was done using disk diffusion method as described by Kirby-Bauer employing CLSI (2017) guidelines. Three colonies of *S. aureus* were dispersed into 5 mls of sterile normal physiological saline and vortexed well. The turbidity was then adjusted to match that of 0.5 Mac Farland standard, which represented the bacterial concentration used.

Sterile cotton swab was used for inoculation of the sample by dipping it into the inoculum suspension and inoculating the sample onto Mueller-Hinton agar (Oxoid LTD, Basingstoke, Hampshire, England) plates by streaking on to the agar and allowed to dry (Adhikari *et al*., 2017**).** The following panel of antibiotics were used; cefoxitin 30µg (as the surrogate antibiotic for methicillin), ampicillin10µg, gentamycin 10µg, ciprofloxacin 5µg, amoxicillin-clavulanic acid 30µg, erythromycin 15µg tetracycline 30µg, trimethoprim/sulfamethoxazole 1.25/23.75µg all purchased from Oxoid LTD, Basingstoke, Hampshire, England. The respective antimicrobial disks were placed on the agar and compacted gently to ensure good total contact with the agar surface. The disks were incubated at 37^{0}C for 24 hours. The zones of inhibition

diameters were determined by calculating an average of two replications of zone diameter reading and interpreting it following the standard break points as given in CLSI (2017). *S. aureus* ATCC 25923 was used as reference positive organism and distilled water (DH_2O) as a negative control (CLSI, 2017) and both were subjected under the same laboratory conditions as the test organism (Enright *et al*., 2002). The standard organism showed the expected zones of inhibition as in the CLSI (2017) guidelines.

3.11.1 Interpretation for Antibiotic susceptibility

Table 3.1: Interpretive category and Zone Diameter Breakpoints (CLSI, 2017)

Antimicrobial Agent	Disk content	Interpretive category and Zone Diameter Breakpoints (Nearest Whole mm)		
		Susceptible	**Intermediate**	**Resistant**
Cefoxitin	**30 µg**	**≥ 22**	**-**	**≤ 21**
Ampicillin	**10 µg**	**≥ 17**	**-**	**≤16**
Ciprofloxacin	**5 µg**	**≥ 21**	**16-20**	**≤ 15**
Erythromycin	**15 µg**	**≥ 23**	**14-22**	**≤ 13**
Gentamycin	**10 µg**	**≥ 15**	**13-14**	**≤ 12**
Tetracycline	**30 µg**	**≥ 19**	**15-18**	**≤ 14**
Amoxicillin/Clavulanic Acid	**30 µg**	**≥ 20**	**-**	**≤ 19**
Sulfamethoxazole/Trimethoprim	**25 µg**	**≥ 16**	**11-15**	**≤ 10**

The zone diameters of inhibition around the disk for various antimicrobial agents were measured to the nearest whole millimetre and the interpretive category of each antibiotic was determined using the criterion described by CLSI (2017) (**Table 3.1**).

S. aureus from both animals and human isolates that demonstrated resistance to cefoxitin were sub cultured on Tryptone Soya Agar and incubated at 37 ^{0}C for 18-24 hours. The pure colony

of *S. aureus* was harvested and stored in cryovial tubes containing 10 % w/v skimmed milk at – 20 ^{0}C awaiting DNA extraction for molecular characterization.

3.11.2 Detection of MRSA by disk diffusion

Determination of methicillin resistant *S. aureus* was done through antibiotic susceptibility testing using cefoxitin 30μg. If the clear zone diameter growth inhibition was equal or less than 21 mm nearest to whole millimetres, as per the interpretive category and zone diameter breakpoints (CLSI, 2017), the culture was interpreted and reported as MRSA positive.

3.12 Molecular identification of *S. aureus* and PCR Detection of resistant genes

The isolates that were resistant phenotypically to cefoxitin 30μg by disk diffusion method were genotypically analyzed by PCR, sequencing and Blast analysis. Genes encoding for resistance to cefoxitin (*mec*A and *mec*C), fluoroquinolone (*gyr*A and *gyr*B) and tetracyclines (*tet*M) were analysed by polymerase chain reaction (PCR) using specific primer pairs (**Table 3.2**). The PCR products were separated in 1.5 % agarose gels by electrophoresis stained with ethidium bromide and visualized using A UV trans-illuminator digital camera (Gelmax 125 imager, Cambridge UK) with a UVP software interphase computer (Upland CA, USA).

Table 3.2: Primers used in the detection of thermo-nuclease (*nuc*) gene, *mec*A, *mec*C,*gyr*A, *gyr*B and *tet*M genes

Primer	Sequence (5'-3')	Target gene	Amplicon size (bp)	Reference source
***mec*A(F)** ***mec*A(R)**	AAAATCGATGGTAAAGGTTGGC AGTTCTGGAGTACCGGATTTGC	*mec*A gene	533 bp	Pournajaf *et al.,* 2014
***mec*C-F** **mecC-R**	GAAAAAAAGGCTTAGAACGCCTC CCTGAATCTGCTAATAATATTTC	*mec*C gene	718 bp	Stegger *et al.,* 2012
SA- (F) **SA-(R)**	GCGATTGATGGTGATACGGTT CAAGCCTTGACGAACTAAAGC	*nuc* gene	276 bp	Wang *et al.,* 1997
***gyrA*(F)** ***gyrA*(R))**	ACGCAAGAGAGATGGTT TCAGTATAACGC ATCGC AGC	*gyr*A gene	270 bp	Chatur *et al.,* 2014
***gyrB*(F)** ***gyrB*(R)**	ATGGCAGCTAGAGGAAGAGA GTGATCCATCA ACATCC GCA	*gyr*B gene	382 bp	Chatur *et al.,* 2014
***tet*M (F)** ***tet*M (R)**	GAGGTCCGTCTGAACTTTGCG AGAAAGGATTTGGCGGCACT	*tet*M gene	580bp	Zhang *et al.,* 2012

3.12.1. Extraction of DNA

Bacterial DNA was extracted by the boiling technique in which pure colony of *S. aureus* was put into an Eppendorf tubes containing 500µl of double distilled water, homogenized by vortexing for one minute, and then boiled at 100 ^{0}C for 30 minutes in a water bath. After boiling and cooling, the products were centrifuged at 15000 rpm for 10 minutes. The supernatant containing DNA was obtained and kept at -20 ^{0}C until used as a DNA template for **PCR** (Jahan *et al*., 2015).

3.12.2 Confirmation of *S. aureus* isolates

PCR reaction was performed to amplify *thermo-nuclease* (*nuc*) gene of all strains of *S. aureus* using specific primer pair. The PCR for amplification of *nuc* gene was performed as described

by Wang *et al*., (1997), with minor modifications. A volume of 8 µL of prepared DNA (0.5 µg) was titrated to a final volume of 20 µL PCR mixture containing 10 µL of 2x Master Mix (Ampliqon, Denmark), including 1x PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA polymerase, (Ampliqon Co., Denmark), 0.5 µL of 10 µmol/L each primer and 1 µL of sterile distilled water.

The PCR was run in a Veriti 96-well Thermal Cycler (applied Biosystems, life Technology, Singapore). The conditions for amplification were; one cycle of 94 °C for 3 seconds, then 35 cycles of 94 °C for 3 seconds, 50 °C for 10 seconds and 72 °C for 35 seconds at the transition speed S-9, and finally, one cycle of 72 °C for 1min and 45 °C for 2 seconds. The PCR products were separated by electrophoresis in 1.5 % agarose gels containing ethidium bromide (10 mg ml^{-1}).

3.12.3 Detection of *mec*A gene by PCR technique

The *mec*A gene identification and amplification was done in Molecular Biology Laboratory, Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya. Detection of *mec*A gene was done according the method described by Pournajaf *et al*., (2014), with minor modifications. PCR was done in Veriti 96-well Thermal Cycler (applied Biosystems, life Technology, Singapore). A volume of 5 µL of prepared DNA was added to a final volume of 20 µl containing 10 µL of 2x Master mix (Ampliqon, Denmark), containing 1x PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA polymerase, (Ampliqon Co., Denmark), 0.7 µL of 10 µmol/L each primer and 3.6 µL of sterile distilled water.

The thermal cycling process for PCR comprised of 95 °C for 3 minutes, followed by 33 cycles of 95 °C for 41 seconds, 53 °C for 30 seconds and 72 °C for 1minute, with a final extension at 72 °C for 6 minutes. The amplification products were visualized by electrophoresis in 1.5 % agarose gels stained with ethidium bromide under UV illumination. The visualization of the 533bP band signified isolates with *mec*A gene.

3.12.4 Detection of *mec*C gene by PCR technique

Identification and amplification of *mec*A_{LGA251} among the phenotypically resistant mecA negative isolates of *S.aureus* was done in Molecular Laboratory, Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya

The detection of mecC was carried out as per the protocol described by Stegger *et al*., (2012), with minor modifications. PCR was run in Veriti 96-well Thermal Cycler (applied Biosystems, life Technology, Singapore). A volume of 5 µL of prepared DNA was titrated to a final volume of 25µl containing 12.5 µL of 2x Master mix (Ampliqon, Denmark), including 1x PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA polymerase, (Ampliqon Co., Denmark), 2 µL of 10 µmol/L each primer and 3.5 µL of sterile distilled water. The PCR products were then visualized through electrophoresis in 1.5 % agarose gels stained with ethidium bromide under UV illumination. The visualization of the 710 bP bands indicated the isolates that harbour the *mec*C gene.

3.12.5 Detection of *gyr*A and*gyr*B gene by PCR analysis

The detection of *gyr*A and *gyr*B genes was undertaken according to the method previously described by Chatur *et al.*, (2014) with minor modifications. PCR was done in Veriti 96-well Thermal Cycler (applied Biosystems, life Technology, Singapore). The reaction mixture was

optimized to contain a final volume 25 µL of 2 X master mix (Ampliqon, Denmark), containing 0.05 unit/ µl Taq DNA Polymerase, reaction buffer, 4 mM MgCl2, 0.4 mM of each dNTP, 10pmole of each forward and reverse primer (10pmole/µl), 18.0 µl nuclease free distilled water and 5 µl of DNA template for both the genes. The PCR reaction was performed at annealing temperature of 45 ^{0}C and 53 ^{0}C for 50 seconds, respectively. Rest of reaction was set as, Initial denaturation at 95^0 C for 5 minutes, 35 cycles of denaturation at 95 ^{0}C for 50 seconds, extension at 30 seconds and final extension at 72^{0}C for 7 minutes.

3.12.6 Detection of *tet*M gene by PCR analysis

Genes encoding for resistance to tetracyclines (*tet*M), were used for the analysis. The primers outlined in Table 1 used for PCR amplification in a 50 µL PCR mixture in a reaction previously described by Zhang *et al.,* (2012). The PCR products were separated in 1.5% agarose gels by electrophoresis stained with ethidium bromide and visualized using A UV trans-illuminator digital camera (Gelmax 125 imager, Cambridge UK) with a UVP software interphase computer (Upland CA, USA).

3.13 Gel Electrophoresis of PCR products

Agarose gel 1.5% w/v in 0.5X tris boric ethylene-diamine-tetra-acetic sodium (TBE) containing 0.5µg/ml ethidium bromide was prepared and melted in 0.5X TBE using a microwave. The prepared gel was then caste and allowed to solidify. A volume of 10µl portion was mixed with 2µl of 6X gel loading dye and loaded into electrophoresis gel wells while submerged in 1X TBE running buffer. A 100bp DNA ladder molecular marker (England biolabs) was loaded in one of the wells. Also included were a known positive (*S.aureus*) DNA and a negative control (DNAse free distilled water). A constant voltage of 10V/cm was applied and amplified fragments were allowed to migrate until appropriate band separation was

achieved. A UV trans illuminator digital camera (Gelmax 125 imager, Cambridge UK) with a UVP software interphase computer (Upland CA, USA) was used to visualize DNA bands relative to the molecular weight marker.

3.14 Sequencing of Resistant genes

Purified PCR products that harboured the resistant gene after being identified through amplification of *mec*A gene and *mec*C alongside with their specific primers both forward and reverse previously used for PCR detection and identification of resistant genes were submitted to Humanizing Genomics, Macrogen Europe Laboratory- Netherlands for oligonucleotide sequencing.

3.15 DNA sequencing of PCR products

In order to determine the micro-organism (S. aureus) and the resistant genes, *nuc* gene (276-bp), *mec*A (533-bp) and *mec*C (718-bp), genes were amplified, and the nucleotide sequences of the amplified products were subsequently determined commercially (Macrogen, Netherlands). Sequences were obtained in ABI files that were opened and edited to remove unspecific ends using Bioedit version 7.0.4 (Hall, CA, USA) software. Clean sequences were then submitted to NCBI Genbank database (http://blast.ncbi.nlm.nih.govblast.cgi/) and BLASTn program used to test for homology and genetic identity of bacterial isolates.

3.16 Data analysis

Data analyses for phenotypic and genotypic *S. aureus* resistance were performed using the STATA version13 software after being entered into Ms Excel spreadsheet. Descriptive statistics were used on quantitative data. The data was presented in form of tables and charts.

Resistant gene from animal isolates (milk) and human isolates (blood) were linked at genetic level by analysing target sequences for similarities.

Basic local alignment search tool (BLASTn) was used to calculate statistical significance on regions of similarity between the current study bacteria isolates sequences and that of national centre of biotechnology information (NCBI) sequence database.

3.17 Ethical issues

Ethical approval for this study was sort for and granted by the University of Nairobi's Faculty of Veterinary Medicine's Biosecurity, Animal Use and Ethics Committee (REF: FVM BAUEC/2021/301).

Informed consent was sought from the cow owners prior to sample collection. Consent to collect samples from the University Dairy Animal farm and Ngong government veterinary farm was requested and granted by the Chairman of the Department and farm's managers respectively.

CHAPTER FOUR: RESULTS

4.0. *S. aureus* isolation and identification

4.1 Culture and biochemical characteristics of dairy milk samples

Out of 353 milk samples, cultured on Mannitol Salt Agar (MSA), a total of 171(48.4%) showed a characteristic small, circular, cocci smooth yellow colonies on MSA with a colour change on MSA media from phenol red to yellow and therefore the colonies were presumed to be *S. aureus* (Figure 3A). Out of 171 isolates of *S. aureus* from milk that were cultured on Sheep Blood Agar (SBA), 91(25.8%) showed colonies with a smooth appearance, convex in shape, cream white in colour and a clear zone around the colonies, indicating a positive β-haemolytic effect of *S. aureus* on SBA (Figure 3B). All the 91 *S.aureus* isolates cultured on Tryptic Soy Agar (TSA) showed a characteristic growth of round cream white colonies on TSA, which is a positive indication of successful enrichment of *S. aureus.* All the 91(100%) isolates showed an active bubbling on reaction with 3%v/v hydrogen peroxide on slide catalase test, indicating a positive presumptve isolates of *S. aureus* (Figure 4B). On coagulase test, 52 (14.7%) out of 91 isolates showed a phenomenal clotting with varied level of coagulation ranging from a loose clot suspended in plasma to semi-solid immovable clot examined by tilting each test tube gently hence a positive coagulase test indicating isolates of *S. aureus* (Figure 4A).

4.2. Culture and biochemical characteristics of human blood samples

Out of 142 human blood samples that were cultured on Mannitol Salt Agar (MSA), only 140 (98.6%) showed a characteristic small, circular, smooth yellow colonies on MSA with a colour change on media from phenol red to yellow and therefore the colonies were presumed to be *S. aureus* (**Fig.3A**). All the 140 *S. aureus* isolates were then cultured on Sheep Blood Agar (SBA)

and 112 (78.9%) showed a clear zone around the colonies, indicating a positive β-haemolytic effect of *S. aureus* on SBA (**Fig.3C**). All the 112 (100%) isolates showed an active bubbling on reaction with 3%v/v hydrogen peroxide on slide catalase test, indicating a positive presumptve isolates of *S. aureus* (**Fig. 4C**). Out of 112 isolates from human blood, 56 (39.4%) showed a phenomenal clotting with varied grades of coagulation hence a positive coagulase test indicating isolates of *S. aureus* (**Fig. 4A**). Observations from culture and biochemical test shows that 56 (39.4%) isolates were confirmed as positive *S. aureus* (**Table 4.1**).

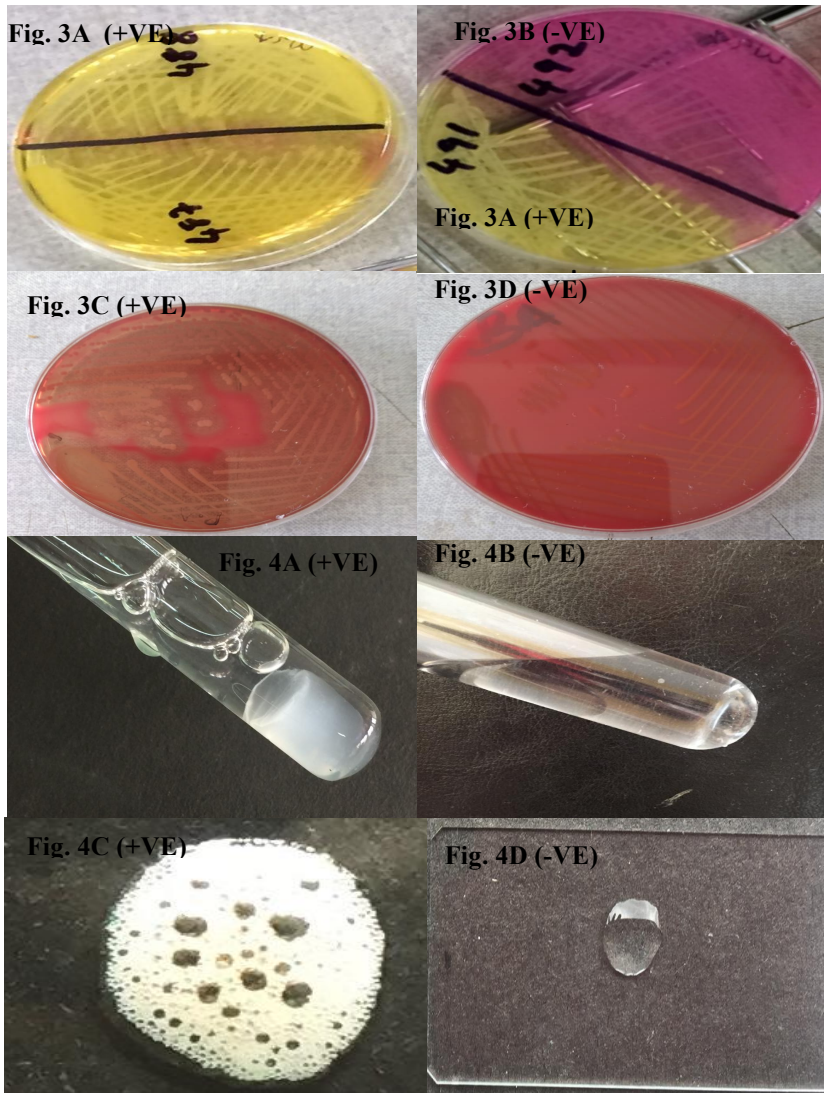


Fig. 3A- 4D: Isolation and identification of *S. aureus* suspects

Table 4.1: *S. aureus* isolates recovered on culture and confirmed as positive isolates of *S. aureus* using biochemical test

Cattle milk and Human blood samples

Sample Source	No. of Samples (N)	Growth on MSA (n%)	β-Haemolysis	Growth on TSA (n%)	Catalase test (n%)	Coagulase test (n%)
Animals (Cow Milk)	**353**	**171(48.4)**	**91(25.8)**	**91(100)**	**91(100)**	**52(14.7)**
Human (Blood)	**142**	**140(98.6)**	**112(78.9)**	**112(100)**	**112(100)**	**56(39.4)**

MSA-Mannitol Salt Agar, TSA- Tryptic Soy Agar,

4.2. Confirmation of *S. aureus* isolates by PCR

Using specific primer pair (Table 3.2), PCR reaction was performed on representative samples of the presumed isolates of *S. aureus* to rule out any mendacious positive results of *S. aureus* by the use of control primer pair targeting the amplification of 276bp amplicon size of nuclease gene.

Out of the 52 (14.7 %) phenotypically positive isolates of *S. aureus* obtained from raw cow milk and 56 (39.4%) from human blood through the conventional method, 26 (7.4 %) and 53(37.3%) were confirmed to be *S. aureus* through the amplification of the presence of the characteristic *nuc* gene by PCR (**Fig.4.2**)

Fig. 4.2: PCR amplification of nuclease gene

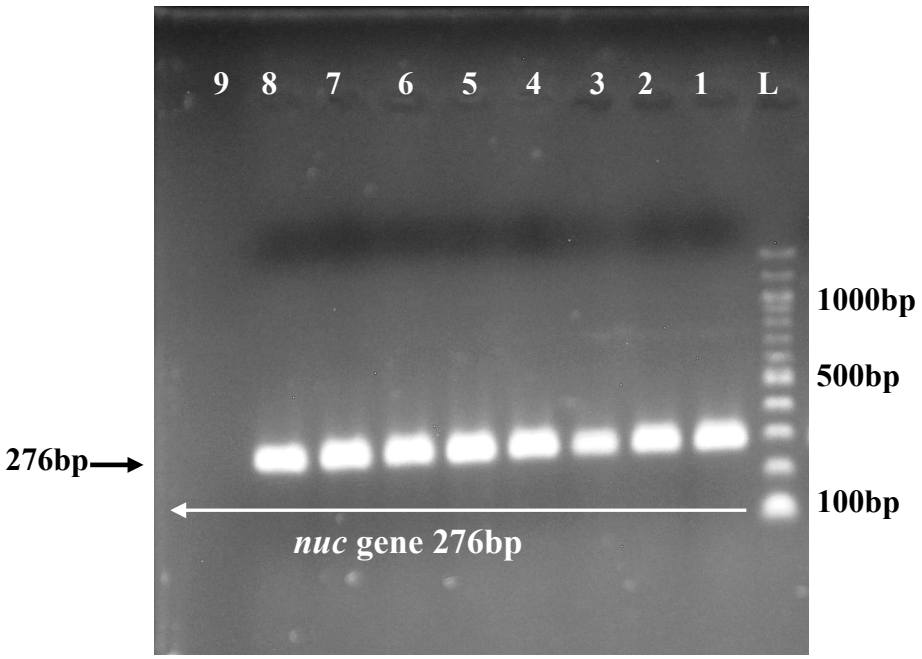


Fig. 4.2. Ethidium bromide stained 1.5% w/v agarose gel electrophoresis of *S. aureus* PCR products and 100-bp ladder. From right to Left, Lane 1- positive sample (*S. aureus*, ATCC 25923); Lane 9- negative sample (purified water); Positive *S. aureus* in Lane 2 to 8 and L is 100-bp ladder.

BLASTn results of the nucleotide sequences alignment confirmed that sequences were homologous to *S. aureus.* The sequences alignment of the representative samples with the BLASTn showed a 99 % nucleotide identity for isolates 80SAR, 115SAR, 476SAR, 479SAF and 493SAF whereas isolates 469SAF, 471SAR, 484SAF and 491SAR showed nucleotide percentage identity of 100 % and isolate 487SAR showed nucleotide percentage identity of 96% (Table 4.3).

Using BLASTn analysis, isolate sequences that showed amplicon size of 276bp were confirmed to belong to *S. aureus* as their homology.

The PCR sequenced products that were confirmed by BLASTn analysis to belong to *S. aureus* as their homologue were assigned accession numbers for the purpose of identification (Table 4.2)

Table 4.2: Nuclease genes of *S. aureus* and their sequenced homologue and identity obtained from NCBI genebank using Nucleotide-nucleotide BLASTn.

Isolate information			**Alignment information (NCBI genebank)**	
Isolate ID	Target gene	Homologue	E Value	Nucleotide % identity
80 SAR	*nuc* gene	*S. aureus*	2e-116	99%
115 SAR	*nuc* gene	*S. aureus*	3e-120	99%
469 SAF	*nuc* gene	*S. aureus*	2e-117	100%
471 SAR	*nuc gene*	*S. aureus*	2e-117	100%
476 SAR	*nuc* gene	*S. aureus*	3e-120	99%
479 SAF	*nuc* gene	*S. aureus*	7e-118	99%
484 SAF	*nuc* gene	*S. aureus*	2e-117	100%
487 SAR	*nuc* gene	*S. aureus*	2e-108	96%
491 SAR	*nuc* gene	*S. aureus*	5e-118	100%
493 SAF	*nuc* gene	*S. aureus*	5e-116	99%

4.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done on all the 26 isolates of *S. aureus* from animals (raw cow milk) and 53 isolates from human blood. Percentage resistance for each antibiotic was calculated as a proportion of isolates resistant (n), to the total number (N) of isolates multiplied by hundred.

4.3.1 Susceptibility patterns of *S.aureus* isolates from raw cow milk and human blood

The susceptibility of isolates was categorized as resistant, intermediate resistant or susceptible to eight panels of antibiotics (**Table 4.3**).

4.3.1.1. Cattle Milk isolates

All the 26 (100%) isolates from raw cow milk showed susceptibility to ciprofloxacin and amoxicillin-clavulanic acid but three isolates of *S.aureus* were resistant to cefoxitin (11.54%), four isolates demonstrated resistance to ampicillin (15.38%). One s. aureus isolate was resistant to erythromycin (3.85 %) whereas two isolates showed intermediate resistance to gentamycin (7.69 %). Four (15.38 % isolates of *S.aureus* showed resistance to tetracycline (15.38%) and 1 (3.84 %) isolates showed resistance to sulfamethoxazole-trimethoprim with an intermediate resistance of 1 (3.85 %).

4.3.1.2. Human blood isolates

Antimicrobial susceptibility of (53) *S.aureus* isolates from human blood samples demonstrated a varied resistance patterns against an array of eight antimicrobial agents from different antibiotic classes. The resistance to cefoxitin was 20 (37.74%), ampicillin 27 (50.94%) ciprofloxacin 15 (28.3%), erythromycin 10 (18.87%) with an intermediate resistance of 9 (16.98%), gentamycin 15 (28.30%) with an intermediate resistance of 1 (1.89%), tetracycline 19 (35.85), amoxicillin-clavulanic acid 15 (28.30%) and sulfamethoxazole-trimethoprim 29 (54.72%) with an intermediate resistance of 2 (3.77%) respectively (**Table 4.3**).

Table 4.3: Resistance of *S.aureus* isolates from raw cattle milk and human blood to eight different antimicrobial agents

	ANIMALS (Cow Milk) (N=26)			HUMANS (BLOOD) (N=53)		
ANTIBIOTICS	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Cefoxitin 30µg	3(11.54)	0	23(88.46)	20(37.74)	0	33(62.26)
Ampicillin 10µg	4(15.38)	0	22(84.62)	27(50.94)	0	26(49.06)
Ciprofloxacin 5µg	0	0	26(100)	15(28.3)	0	38(71.70)
Erythromycin 15µg	1(3.85)	2(7.69)	23(88.46)	10(18.87)	9(16.98)	34(64.15)
Gentamycin 10µg	2(7.69)	0	24(92.31)	15(28.3)	1(1.89)	37(69.81)
Tetracycline 30µg	4(15.38)	0	22(84.62)	19(35.85)	0	34(64.15)
Amoxicillin/ Clavulanic acid 30µg	0	0	26(100)	15(28.3)	0	38(71.7)
Sulfamethoxazole/ Trimethoprim 25µg	1(3.84)	1(3.85)	24(92.31)	29(54.74)	2(3.77)	22(41.51)

Key; R-Resistant, I- Intermediate resistant, S- Susceptible

4.3.2 Resistance of *S.aureus* isolates from raw cattle milk and human blood to β-lactam and to non β-lactam antibiotics

4.3.2.1. Milk samples

Isolates from cow milk showed resistance pattern of 4/26 (15.38% to ampicillin, cefoxitin 3/26 (11.54%) and none of the isolate showed resistance to amoxicillin-clavulanic acid (0 %).

Resistance to non- non-β lactam antibiotics to isolates from raw cow milk showed varied susceptibility patterns with all the 26 isolates being susceptible to ciprofloxacin while erythromycin showed resistance of 11.54%, gentamycin (7.69%), tetracycline (15.38%) and Sulfamethoxazole-trimethoprim (7.69%.)

4.3.2.2. Human isolates

Isolates from human blood showed resistance pattern of 27 (50.94%) to ampicillin, cefoxitin 20 (37.74%) and 15 (28.30%) to amoxicillin-clavulanic acid. To non-β lactam antibiotics, higher resistances were observed for Sulfamethoxazole-trimethoprim (58.49%), followed by tetracycline and erythromycin (35.84%) respectively and gentamycin showed resistance of 30.19% (**Table 4.4**).

Table 4.4 Resistance pattern of *S.aureus* isolates from raw cow milk and human blood to some β-lactams and to non β-lactam antibiotics

Number of resistant *S. aureus* isolates				
	Animals (N=26)		Humans (N=53)	
Antibiotic category	n	(n %)	n	(n %)
a). β-Lactam antibiotics				
Cefoxitin	3	11.54	20	37.74
Ampicillin	4	15.38	27	50.94
Amoxicillin/clavulanic acid	0	0	15	28.30
b). Non β-lactam antibiotics				
Ciprofloxacin	0	0	15	28.30
Erythromycin	3	11.54	19	35.84
Gentamycin	2	7.69	16	30.19
Tetracycline	4	15.38	19	35.84
Sulfamethoxazole/trimetho.	2	7.69	31	58.49

4.3.3. Resistant phenotypes of *S. aureus* isolates from raw cow milk and human blood to eight (8) different classes of antimicrobial agents

4.3.3.1. Milk samples

Seven resistant phenotypes were evident; TET (7.69%), FOX-GENT (3.85%), AMP-TET (3.84%), ERY- SXT (3.84%), AMP (11.54%), FOX (7.69%), and GENT-TET (3.85%) wherein; AMP and FOX and TET were the top three frequently identified phenotypes. None of the isolates exhibited resistance to ≥3 categories of antimicrobials (no multidrug resistance observed) (**Table 4.6**).

4.3.3.2. Human samples

Greater diversity among resistance phenotypes for *S. aureus* from human blood was noted in this study. Twenty-two (39.28%), isolates showed resistance to at least one antimicrobial class, 9 (16.07%) isolates showed resistance to 2 antimicrobial classes, 4 (7.14%) isolates showed resistance to 3 antimicrobial categories, one (1.79%) isolate displayed resistance to 5 different classes of antimicrobials, 9 (16.07%) isolates, were resistant to 6 antimicrobial categories, one (1.79%) isolate showed resistance to 7 different antimicrobial classes and 4 (7.14%) isolates displayed resistance to all the eight(8) antimicrobial categories.

Ninteen resistant phenotypes of *S. aureus* isolates from human blood in the current study were identified as; TET (15.09%), SXT (13.20%), AMP (9.43%), FOX (3.77%), AMP-TET (3.77%), TET-SXT (5.66%), ERY-SXT (3.77%), FOX-AMP (3.77%), ERY-TET-SXT (1.88%), AMP-ERY-SXT (1.88%), AMP-TET-SXT (1.88%), FOX-AMP-SXT (1.88%), FOX-AMP-CIP-GENT-AMC (1.88%), FOX-AMP-CIP-ERY-GENT-AMC (1.88%), FOX-AMP-

CIP-ERY-GENT-AMC (1.88%), FOX-AMP-CIP-ERY-GENT-AMC-SXT (1.88%) and FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT (7.54%) wherein 9 profiles with nineteen 19 (35.84 %) isolates displayed resistance to ≥ 3 antimicrobial categories therefore multidrug resistance (MDR) (**Table 4.5**)

Table 4.5. Proportion of antimicrobial resistant phenotypes of *S. aureus* isolates from human blood, including multidrug resistant *S. aureus*

Antimicrobial resistant Phenotypes	Phenotype Proportion (n%)	Type of Resistance
FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT	4(7.55)	MDR
FOX-AMP-CIP-ERY-GENT-AMC-SXT	1(1.88)	MDR
FOX-AMP-CIP-GENT-AMC-SXT	8(15.09)	MDR
FOX-AMP-CIP-ERY-GENT-AMC	1(1.88)	MDR
FOX-AMP-CIP-GENT-AMC	1(1.88)	MDR
FOX-AMP-SXT	1(1.88)	MDR
AMP-TET-SXT	1(1.88)	MDR
AMP-ERY-SXT	1(1.88)	MDR
ERY-TET-SXT	1(1.88)	MDR
FOX-AMP	2(3.77)	
ERY-SXT	2(3.77)	
TET-SXT	3(5.66)	
AMP-TET	2(3.77)	
FOX	2(3.77)	
AMP	5(9.43)	
SXT	7(13.20)	
TET	**8(15.09)**	

Key; FOX (cefoxitin), AMP (ampicillin), CIP (ciprofloxacin), ERY (erythromycin), GENT (gentamycin), TET (tetracycline), AMC (amoxicillin-clavuranic acid), SXT (sulfamethoxazole-trimethoprim), MDR (Multidrug resistant).

4.4. Genetic determinants responsible for antimicrobial resistance phenotypes

In this study, five genes were investigated for genotypic characterization of MRSA phenotypes using gene specific primers (**Table 3.2**). Out of 20 isolates of *S.aureus* isolated from human blood that were phenotypically resistant to cefoxitin, 17 (85 %) isolates expressed *mec*A gene

and 3 (15 %) isolates that did not express *mec*A nor *mec*C gene. Out of the 53 isolates from human blood, 22.64 % expressed *gyr*A gene, 24.53 % expressed *gyr*B gene and 18.87 % expressed *tet*M gene by PCR (**Fig 2C, 2D and 2B**). On the other hand, 4 (15.38 %) of the 26 isolates from raw cow milk expressed *tet*M gene (**Fig. 2D**) and all the three strains of *S. aureus* isolates that were phenotypically resistant to methicillin did not express both the *mec*A and *mec*C genes by PCR assay. The analysis of the sequenced resistant determinants revealed that the genes were harboured by *S. aureus*. The *mec*A gene detected in this study was gene fragment with 533bp amplicon size (**Fig. 4.3**).

Fig. 4.3 Agarose gel electrophoresis of PCR detection of *S. aureus* resistant genes.

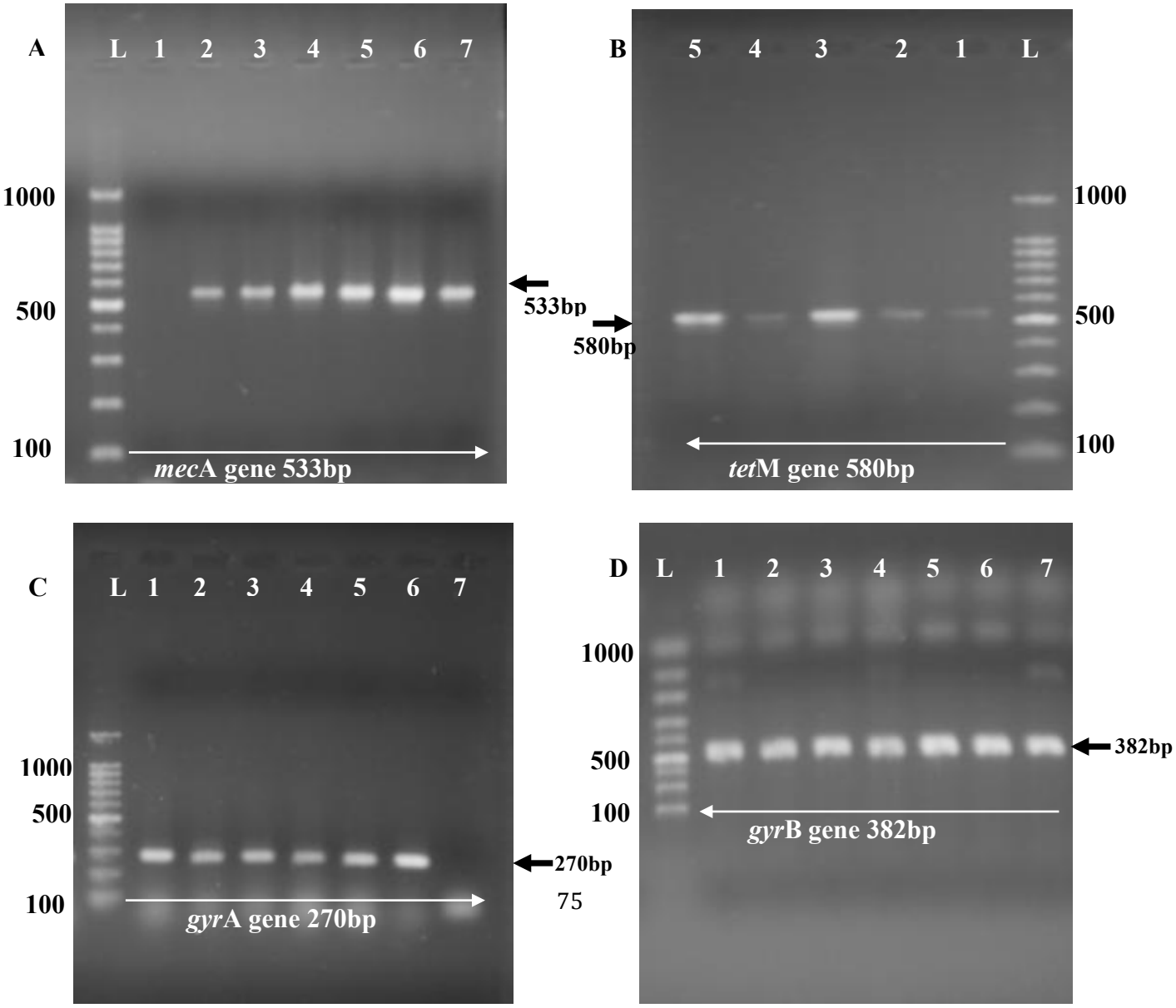


FIg. 4.3. Polymerase chain reaction detection of *S. aureus* resistant genes; (A) *mec*A gene (533 bp), (B) *tet*M gene (580 bp), (C) *gyr*A gene (270 bp), and (D) *gyr*B gene (382 bp).

4.4.1 Multidrug-Resistant genes of *S. aureus* from human blood and animal to different antimicrobial agents

Various resistant phenotypes and the corresponding genetic determinants for resistance of the MDR-*S. aureus* isolates from human blood were detected in our study. About (78.95 %) of the multidrug resistant isolates from human blood were MRSA. About 73.68 % of MDR-*S. aureus* harbored *mec*A, 63.16 % *gyr*A, 68.42 % *gyr*B and 26.32 % *tet*M. Our results indicate that *mec*A gene was the predominant genetic determinant for methicillin resistance phenotypes, followed by *gyr*B, gyrA and *tet*M for the resistance of ciprofloxacin and tetracycline respectively (**Table.4.6**)

Table 4.6 Multidrug-Resistant genes of *S. aureus*

ID	Sample source	Antimicrobial agents	Resistant genes expressed
115	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
469	**Blood**	**FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT**	***mec*A,*gyr*A,*gyr*B,*tet*M**
470	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
471	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
475	**Blood**	**FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT**	***mec*A,*gyr*A,*gyr*B,*tet*M**
476	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
479	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC**	***mec*A,*gyr*A,*gyr*B**
482	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
484	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
487	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
493	**Blood**	**FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT**	***mec*A,*gyr*A,*gyr*B,*tet*M**
494	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A,*gyr*A,*gyr*B**
485	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***gyr*B**
490	**Blood**	**FOX-AMP-CIP-ERY-GENT-AMC-SXT**	***mec*A**
488	**Blood**	**FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT**	***mec*A**
39	**Blood**	**AMP-TET-SXT**	***tet*M**
18	**Blood**	**ERY-TET-SXT**	***tet*M**
491	**Blood**	**FOX-AMP**	***mec*A**
80	**Blood**	**FOX-AMP**	***mec*A**
407	**Milk**	**GENT-TET**	***tet*M**
156	**Milk**	**AMP-TET**	***tet*M**
122	**Milk**	**TET**	***tet*M**
119	**Milk**	**TET**	***tet*M**

Key; FOX (cefoxitin), AMP (ampicillin), CIP (ciprofloxacin), ERY (erythromycin), GENT (gentamycin), TET (tetracycline), AMC (amoxicillin-clavuranic acid), SXT (sulfamethoxazole-trimethoprim)

4.5 BLAST analysis of DNA sequences

4.5.1 Identification of DNA sequences for resistant gene

Sequence analysis by BLASTn tool revealed that the entire representative MRSA isolates harbored the resistant *mec*A gene and sequences were homologous to *S.aureus*.

The sequences alignment with the BLASTn showed a significant similarity of 99 % for isolates 469R, 487R and 493R. Isolates 471R, 476R, 491R and 494F showed 98 % nucleotide identity

while isolates 484R, 479R, 80R and 115R showed 97 %, 96 %, 91% and 90 % identity respectively. Isolates 469R, 471R, 476R, 484R, 487R, 491R and 494F showed no nucleotide gaps with an E- value of 0 upon alignment whereas isolates 80R and 115R showed 3 % and 2 % nucleotide alignment gaps respectively (**Table 4.7**)

Table 4.7: MRSA genes from selected isolates, their sequenced homologue and identity obtained from NCBI genebank using Nucleotide-nucleotide BLASTn .

Isolate information

Isolate ID	Target gene	Homologue	E value	Nucleotide % Identity
80R	*mec*A gene	*S. aureus*	4e – 18	91%
115R	*mec*A gene	*S. aureus*	3e – 138	90%
469R	*mec*A gene	*S. aureus*	0	99%
470R	*mec*A gene	*S. aureus*	9e - 64	98 %
471R	*mec*A gene	*S. aureus*	0	98%
476R	*mec*A gene	*S. aureus*	0	98%
479R	*mec*A gene	*S. aureus*	5e -127	96%
484R	*mec*A gene	*S. aureus*	0	97%
487R	*mec*A gene	*S. aureus*	0	99%
491R	*mec*A gene	S. aureus	0	98%
493R	*mec*A gene	*S. aureus*	5e -137	99%
494F	*mec*A *gene*	*S. aureus*	0	98%

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

S. aureus is the leading causal agent for both healthcare and community associated ailments and probably the single most frequent cause of healthcare affiliated infections globally (McDonald, 2006; Fluit *et al*., 2001and Lowy, 1998). In human and livstock health, *S. aureus* is considered significant causal agent of zoonotic infection and possible transmission of MRSA between humans and livestock through various routes can occur (Wang *et al*., 2018).

Whilst antibiotic resistance is de novo phenomenon, it's frequently accelerated as an effect of the bacterium's adaption to indiscriminate antimicrobial use in humans, animals, and the wide usage of disinfectants in farms and at household level (Walsh, 2000). Evolution of MRSA is multifaceted, including host factors, poor initiatives in infection control and antibiotic overuse (Byarugaba, 2010).

In the current study, the prevalence of *S.aureus* in dairy milk was 7.4 %, which was slightly in agreement with the findings of a similar study carried out at the northern pastoral region of Kenya by Omwenga *et al.,* (2019), who reported prevalence of 9.64% in individual lactating dairy animals. The finding in this study was slightly higher compared to Dai *et al*., (2019) who reported 3.9 % in China, Jans *et al*., (2017) reported 67 % in Kenya, 11 % in Somali and 21 % in Ivory Coast. However, Ombui (1992) reported 4.17 %, much lower than previous similar studies by Ngatia (1988), Shitandi and Sternesjö (2004) who reported a prevalence of *S.aureus* of 55% and 30.6% respectively in Kenya. Asiimwe *et al*., (2017), reported a prevalence of 20.3% in bulk milk and 12.1% in sour milk in Uganda, which was slightly higher than observtions in this study. In Bangladesh, Jahan *et al.,* (2015) reported *S. aureus* prevalence of 25.53%, which was significantly higher than the present study. In South Africa, Ateba *et al.,*

(2010), reported 100 % prevalence of *S. aureus* in raw milk whereas in China Wang *et al*., (2018) reported 46.2% and in Algeria Matallah *et al*., (2019) reported 31.56%, which were higher than the finding in the current study.

Whilst, the *S.aureus* prevalence may vary in respect to size and geographic zone from which sampling was done, a high percentage of these microorganism in milk could be related to low standard of hygienic practices (Ateba *et al*., 2010). The low prevalence of *S. aureus* in milk reported in the current study as compared to previous studies; Wang *et al*., (2018), Jans *et al*., (2017), Jahan *et al.,* (2015) and Ateba *et al.,* (2010) may be due to proper hygiene and good farm management practices among the farms in this study area as was evidenced during the sampling period.

The prevalence of non- duplicate *S. aureus* isolated from human blood in this current study was found to be 37.3 %. The finding was in agreement with a study done in Gabon 34 % and Côte d'Ivoire 32.4 % (Schaumburg *et al.,* 2014) but lower compared to that reported in two in-patient hospitals of 6.3 % (Omuse *et al*., 2014) and 10.1 % in one in-patients level 5 hospital (Aiken *et al*., 2014) in Kenya. In Asmara, Eritrea, Garoy *et al*., (2019), reported a prevalence of 63.1%, among patients which is higher than the one observed in the current study.

These disparities in *S. aureus* prevalence in the current study and that reported from different countries or regions in previous studies may be attributed to repeat cross sectional sampling design, time dependent bias embraced by performing multiple rounds of sampling in patients with longer in-patient stay (Aiken *et al*., 2014). Overally, *S. aureus* is a major blood stream

pathogen with a prevalence ranging between 9.5% and 39 % in most African countries (Schaumburg *et al.,* 2014) and our obseravtion was within this range.

Surveillance on *S. aureus* resistance patterns to various antimicrobials is crucial in understanding susceptibility trends. In this study, antimicrobial resistance pattern was observed in eight different antibiotic categories. Antimicrobial agents tested included; cefoxitin, ampicillin, ciprofloxacin, erythromycin, gentamycin, tetracycline, amoxicillin/Clavulanic acid and sulfamethoxazole/trimethoprim.

In the current study, cefoxitin resistance screening for isolates from raw cow milk revealed 11.54 % MRSA which was in agreement with 15.6 % reported by Ombui *et al* (2000) who carried an evaluation on antibiotic resistance profile and plasmid pattern of *S. aureus* in milk and meat in Kenya. The finding in the current study agree to that reported by Ateba *et al.,* (2010) in South Africa which had resistance of 3.8 % to 16% among commercial farms but was significantly low compared to 64 % to 100 % resistance in communal farms. In this study, resistance to cefoxitin was higher as compared to 7.8 % reported by Shitandi and Sternesjö (2004), 4.97 % by Sudhanthiramani *et al.,* (2015) and 2.20 % by Matallah *et al*., (2019). *S. aureus* resistance was also higher than those reported in Tanzania, 4.4% (Mohammed *et al*., 2018), Iran, 4.9 % (Jamali *et al.,* 2015) and Uganda, 8.69 % (Asiimwe *et al*., 2017). However our observed resistance was much lower than that documented in China, 100 % (Wang *et al*., 2014), Colombia, 100 % (Herrera *et al.,* 2016) and Nigeria, 29.1 % (Umaru *et al*., 2016). These variations in resistance noted between the current study and that reported from different regions of the world may be due to the type of antimicrobial susceptibility test method used. Most researchers in previous studies have used microdilution assay whereas the current study used cefoxitin disc diffusion assay, a method that has been reported to be 100 % sensitive and 91.6

% specific in determining MRSA (Pourmand *et al*., 2014). Also these variations can be attributed to the fact that the current work utilized ecological samples from the udder as compared to samples from cattle with clinical or sub-clinical mastitis utilized in previously reported studies. Milk is normaly considered safe from bacteria if the dairy animal does not suffer from clinical of sub clinical mastitis.

In this study, *S. aureus* isolates from human blood showed cefoxitin resistance of 37.74 %, which was higher than isolates from dairy milk, but was in agreement with reports from India of 36 % by Kumar *et al*., (2017) and Nepal of 43.1% by Ansari *et al.,* (2014) however, the resistance to cefoxitin observed in this study was lower than previously reported of 84.1% in Kenya (Maina *et al.,* 2013), 53.4% in East Africa, (Wangai *et al*., 2019), but higher than that reported in one hospital in Kenya (Aiken *et al*., 2014).

In the current study, the resistance was higher than that reported in Côte d'Ivoire of 5.3 %, Gabon 0 % and in Congo of 1.7 % (Schaumburg *et al*., 2014) and 3.7 % (Omuse *et al*., (2014), in two hospitals in Nairobi, Kenya, but lower compared to 64.1 % reported by Obajuluwa *et al.,* (2016).

The variations observed in resistance of *S. aureus* from human blood to cefoxitin reported globally as compared to our current study may be attributed to repeated cross sectional sampling design, adapted by researchers, time dependent bias embraced by performing multiple rounds of sampling in patients with longer in-patient stay (Aiken *et al*., 2014) and also due to overusage of cephalosporins, a case in which a large group of microorganisms have developed resistance against these antibiotics rendering the drug void of pharmarcological activity.

S. aureus from raw cow milk showed 15.4 % resistance to ampicillin. However, a study in South Africa, on commercial farms, resistance to ampicillin was reported at 3.8 % to 16.3 % respectively, and this was in agreement with the findings in the current study (Ateba *et al.,* 2010) but lower than reported in Northern China, 79.6% (Liu *et al*., 2017) India, 74.4 % (Sudhanthiramani *et al*., 2015), in Kenya, 31.3 % (Ombui *et al*., 2000), in Ethiopia, 64.3% (Daka *et al*., 2012) and in Nigeria, 90.9 %, (Umaru *et al*., 2016). This low level of resistance to ampicillin noted in the current study was very striking as this drug is commonly used for the treatment of staphylococcal infections suggesting that resistance rate for *S. aureus* vary regionally and does not neccessarily be influenced by antibiotic use in veterinary medicine.

With regard to *S. aureus* from human blood, resistance to ampicillin was reported at 50.94 % in the present study. This was lower than 100 % reported in Sudan (Elhassan et al., 2015), 77.2 % in Nigeria (Okonkwo *et al*., 2018), 89.6 % in Owerri (Uwaezuoke and Aririatu, 2005) and 77.3 % in Turkey (Yılmaz and Aslantaş, 2017). Low resistance of *S. aureus* from human blood patients to ampicillin noted in this current study may due to the fact that ampicillin is less commonly used in Kenya to treat enterobacteriaceae related infections because more superior drugs are currently available for the managements susceptible microbes therefore reducing selective resistance strains.

S. aureus isolates from raw cow milk showed 100 % susceptibility to amoxicillin/clavulanic acid. This finding was similar to 100 % susceptibility reported in China (Liu *et al*., 2017) and in Ethiopia 100 % (Mekonnen *et al*., 2018). However, Dai *et al*., (2019), reported resistance of the isolated strains of MRSA to amoxicillin/clavulanic of 8.3%, which was higher than observation in this study. Despite the fact that amoxicillin/clavulanic is frequently used in

treatment and prevention of various animal infections in the study area, the finding in the current work was striking and contrary to high resistance reported by Lemma *et al*., (2021) suggesting that resistance for *S. aureus* vary regionally and does not neccessarily be influenced by antibiotic use in veterinary medicine.

In this study, isolates from human blood showed 28.30 % resistance to amoxicillin/clavulanic acid, which was higher than 10 % reported in India (Kumar *et al*., 2017) and 16.5 % reported by Yılmaz and Aslantaş (2017) in Turkey. The variations in resistance of *S. aureus* from clinical isolates to amoxicillin/clavulanic acid acid may be adoption of different test guideline by different researchers in terms of concentrations of clavulanic acid used which varies by the testing method. The European Committee on Amtimicrobial Susceptibility Testing (EUCAST) method uses a fixed concentration of 2mg/L of clavulanic acid whereas the United States Committee on Antimicrobial Susceptibility Testing (USCAST) or the Clinical Laboratory Standards Institute recommends the use of a fixed ratio between the amoxicillin and clavulanic acid concentration of 2.1mg /L. these different strategies gives different test results and there is no concensus among the committees as to which strategy should be used (Huttner *et al*., 2020) therefore this explains the reason for variations of resistance of *S.aureus* to amoxicillin/clavulanic acid in this current study and that reported from different regions by different researchers.

In current study, all the *S.aureus* isolates from milk showed 100 % susceptibility to ciprofloxacin, which concurs with the reports from Ethiopia (Daka *et al*., 2012), in Uganda (Asiimwe *et al*., 2017) and Nigeria, with 92.7% susceptibility (Umaru *et al*., (2016). However, lower rates were reported in China, 29.6% (Liu *et al*., 2017) and in India, 9.30%

(Sudhanthiramani *et al*., 2015). The high susceptibility of *S. aureus* noted in the current study may be attributed to the fact that ciprofloxacin is significantly important antibiotic in human medicine and is highly restricted for use in the treatment of animal deseases in many countries including Kenya (Mbindyo *et al*., 2021). However, isolates from human blood showed 28.3 % resistance to ciprofloxacin, similar to that accounted in Nigeria of 29.7% (Okon *et al*., 2011). In Sudan, reisatance of 86.5% was reported (Elhassan et al., 2015), 58.4 % in Iran (Pournajaf *et al*., 2014) and 67.1% in Kenya (Maina *et al*., 2013). In most reports, resistance to ciprofloxacin ranged as low as 0% to 42% in some African countries (Lozano *et al*., 2016). It's conceivable that hospital environment serves as an important reservoir of ciprofloxacin resistant *S. aureus*, in the current study, sampling of clinical isolates was only done to outpatients as opposed to in-patients included by many researchers in previous studies.

In this study, 3.85 % *S. aureus* isolates from raw milk were resistant to erythromycin. This low prevalence was similar with 5.2 % reported by Wang *et al*., (2018). However, the findings were lower than reported in China, 46.3 % (Liu *et al*., 2017), in Ethiopia, 32.1% (Daka *et al*., 2012) and in Kenya, 14.6% (Ombui *et al*., 2000). The findings were also lower than resistance rates from Bangladesh of 75 % and 73.3 % by Jahan *et al*., (2015) and Islam *et al*., (2017) respectively. The low resistance of *S. aureus* to erythromycin noted in this study may be related to low use of macrolides tylosin and avoparcin as growth promoters in animals in the study area therefore reduced selective antibiotic pressure and overall reduced selective resistant strain.

About 36 % of *S. aureus* isolates from human blood were resistant to erythromycin. This

finding concurs to large extent with the report by Daka *et al*., (2012) of 32.1% in Ethiopia. However, in this study, About 36 % of *S. aureus* isolates from human blood were resistant to erythromycin. This finding concurs to large extent with the report by Daka *et al*., (2012) of 32.1% in Ethiopia., 58.4 % in Iran (Pournajaf *et al*., 2014) and 55 % in Cameroon (Gonsu *et al*., 2013). The resistance in this study was higher than that recorded in Nigeria of 15.6% by Okon *et al.,* 2011) and 11% in Eritrea (Garoy *at al*., 2019). The African continent reports prevalence ranging from as low as 0 % to 100 % (Falagas *et al* 2013). The high resistance of S. aureus from clinical islotes to erythromycin observed in the current work as compared to that reported by Okon *et al*., (2011) and Garoy *at al*., (2019) may be attributed to indiscriminate prescribing of erythromycin for management of acute respiratory tract infections of viral origin in the study area therefore contributing to erythromycin resistant strain.

About 7.7 % S. *aureus* isolates from raw milk were resistant to gentamycin in this study. This concurs with low resstance of 4.7 % for gentamicin reported in India (Sudhanthiramani *et al*., 2015), in Uganda of 3.3% (Asiimwe *at al*., 2017) and in China of 1.0% (Wang *et al*., 2018). Lower resistances were also reported in Ethiopia of 0% (Mekonnen *et al*., 2018). Resistance to gentamycin in the current work was lower than that documented in Northern China of 11.1 % (Liu *et al*., 2017) and in Nigeria of 23.7 % (Umaru *et al*., 2016). However, Islam *et al*., (2017) and Uwaezuoke and Aririatu, (2005) both reported 100 % and 91.7 % susceptibility for gentamycin respectively. The lower resistance to gentamycin detected in this study could be due to the fact that this study only analyzed raw milk samples from healthy animals as compared to other studies where raw milk from animals with sub clinical or clinical mastitis were analysed.

In this study, Isolates from human blood showed 30.19 % resistance to gentamicin. The findings were close to 35.9 % reported in Nepal (Adhikari *et al*., 2017). Although other studies have reported resistance with gentamicin, in this study, the gentamicin resistance was higher compared to 3 % and 13 % reported in Kenya (Omuse *et al*., 2014 and Gitau *et al*., 2018) respectively, 6.2 % reported in Turkey (Yılmaz and Aslantaş, 2017), 21.6 % and 13.6 % reported in Nigeria among the inpatient and outpatient respectively (Okon et al., 2011), 1.2 % reported in Eritrea (Garoy *et al*., 2019) and 15.4 % reported in Kenya (Kesah *et al*., 2003). Among the aminoglycosides, gentamycin in particular is widely used in Kenya to treat staphylococcal and enterococcal infections and therefore this over use of this agent may explain the observed high resistance of *S. aureus* to gentamycin in this current study.

In the current study, 15.38 % isolates of *S. aureus* obtained from raw cow milk were resistant to tetracycline. This result was in agreement to a previous finding in Kenya of 13.4 % among small-scale milk producers (Shitandi and Sternesjö, 2004), in India of 13.95 % (Sudhanthiramani *et al*., 2015) and 13.0 % in China (Liu *et al*., 2017). However, tetracycline resistance was higher than a previous study in Kenya of 5.5% among large-scale milk producers (Shitandi and Sternesjö *et al*., 2004), in China of 1.4 % (Wang *et al*., 2014) and in Algeria of 5.26 % (Matallah *et al*., 2019). Higher resistance to teracyclines has been reported previously by many investigators like 56.1% in Iran (Jamali *et al*., 2015), in Bangladesh 73.33% (Islam *et al*., 2017), Nigeria 81.8% (Umaru *et al*., 2016), in Uganda of 73.2 % (Asiimwe *et al*., 2017), in Ethiopia of 54.0 % (Mekonnen *et al*., 2018) and 23.9 % in Tanzania (Mohammed *et al*., 2018). The high resistance rates of *S. aureus* isolates from milk to tetracycline in this current work may be due to over use of this drug for treatment and prevention of various animal infectionsin in the study area.

Isolates from human blood showed 35.85 % resistance to tetracycline. Similar resistance was reported in previous studies by many authors like Arabzadeh *et al*., (2018) observed 33.0%, Gitau *et al.*, (2018) 33.2%, Gursoy *et al*., (2009) 34.0% and Bhatt *et al*., (2014) 63.1% to tetracycline. However, this lower resistance has been previously reported by many authors; 63.0% in Iran (Pournajaf *et al*., 2014), 62.0% in Ethiopia, (Deyno *et al*., 2017) and 68.0% in India (Kumar *et al*., 2017). Resistance to tetracycline observed in this study was, however, higher than that reported by Omuse *et al*., (2014) of 15.9 %, Schaumburg *et al*., (2014), of 21.8%, and Yılmaz & Aslantaş, (2017) of 16.5%. The high resistance rates of *S. aureus* isolates to tetracycline in this current work as compared to previously reported studies may be attributed to ease availability and low cost of tetracycline that makes the drug attractive for self medication in developed and developing countries. Equally legislative policies in the country do not effectively prevent the indiscriminate use of tetracycline as animal growth promoters and this virtually determines their resistance patterns (Chopra and Roberts, 2001).

In this study, isolates of *S. aureus* from raw milk showed 3.84 % resistance to trimethoprim/ sulfamethoxazole. This finding is slightly similar to 7.7% reported by Daka *et al.,* (2012), in Ethiopia, 4.65 % (Sudhanthiramani *et al*., (2015). Equally, this finding was slightly higher than an earlier report in Kenya of 2.5% and 3.0 % in large-scale farms and small-scale farms respectively (Shitandi and Sternesjö, 2004). In Iran, Jamali *et al*., (2015) reported 0 % resistance. According to Muloi *et al*., (2019), sulfonamides are the most purchased class of antibiotics by dairy farmaers from agrovet in Nairobi, Kenya and therefore high possibility of misuse of this antimicrobial agent due to self prescription and use and this may contribute to high resistance of this drug as observed in this study.

Isolates from human blood showed 58.51 % resistance to trimethoprim/ sulfamethoxazole. This concurs with findings reported previously in Kenya of 56.9 % (Gitau *et al*., 2018) but higher compared to low resistance rates reported in studies in Côte d'Ivoire of 34.1%, 30.8 % in Congo (Schaumburg *et al*., 2014), in Kenya 42.1% (Omuse *et al.,* 2014), in Iran 44.6% (Pournajaf *et al*., 2014), in Ethiopia 47.0% (Deyno *et al*., 2017) and in Turkey 15.5% (Yılmaz and Aslantaş, 2017). However, in India resistance of 70.0% was reported (Kumar *et al*., 2017) and Adhikari *et al*., (2017) reported 70 % resistance to trimethoprim/ sulfamethoxazole in Napal.

In humans, trimethoprim/sulfamethoxazole is currently used as a prophylactic antimicrobial agent among patients with AIDs/HIV in the prevention of pneumocystis carinii that causes pneumonia in patients with AIDs/HIV infection and therefore selective pressure caused by year long use of trimethoprim/ sulfamethoxazole has been linked to be a driving force to emergence of *S. aureus* resistance trimethoprim/sulfamethoxazole (Olalekan *et al*., 2012). Also the high resistance of *S. aureus* from clinical isolates to trimethoprim/sulfamethoxazole observed in this study may due to the use of sulfadixine-pyrimethamine in the treatment of patients with malaria and this has been linked to a significant increase in the prevalence of *S. aureus* that is resistant to trimethoprim/sulfamethoxazole. These two assumptions may lead one to conclude that the use of sulfonamides for the treatment of patients with non-bacterial infections simultaneously inreases the level of resistance of *S. aureus* to sulfonamides as noted in this study and this can be a serious public health concern (Eliopoulos and Huovinen, 2001)

In the current work, isolates of *S. aureus* from raw cow milk were often resistant to ampicillin and tetracycline (15.38%), followed by cefoxitin and erythromycin (11.54%), then gentamycin and trimethoprim/sulfamethoxazole (7.69%) and all isolates were susceptible to ciprofloxacin and amoxicillin/clavulanic acid (0%). These observations are in conformity with finding of Dai *et al*., (2019), who reported the same trend of resistance, with ampicillin (97%) being the most frequently resistant then erythromycin (50%), gentamycin (33.3%) and lastly trimethoprim/sulfamethoxazole (8.3%). However this result is in disagreement with the report by Umaru *et al*., (2016) who observed ampicillin (90.9%) as the most frequently resistant, followed by tetracycline (81.8%), erythromycin (75.5%), cefoxitin (29.1%), trimethoprim/sulfamethoxazole (27.3%) and gentamycin (23.7%). These wide disparities in antimicrobial resistance among isolated population of *S. aureus* between this study and those reported may be attributed to the fact that many studies have focused more on the resistance pattern of *S. aureus* from a population of dairy animal with clinical or sub-clinical mastitis. In the present work, the resistance pattern *S. aureus* was observed on all dairy cattle with or without clinical or sub-clinical mastitis.

High resistance of *S.aureus* isolates from human blood in the present study were observed against trimethoprim/sulfamethoxazole (58.51%), followed by ampicillin (50.94%), cefoxitin (37.74%), erythromycin and tetracycline (35.85%), gentamycin (30.19%) and least to ciprofloxacin and amoxicillin/clavulanic acid (28.30%). The high resistance noted against trimethoprim/sulfamethoxazole in this study is consistent with similar reports in other African countries and this has been related to overexposure in hospital settings where it is extensively used for prophylaxis against opportunistic infections among HIV patients (Maina *et al*., 2016; Mandomando *et al.,* 2010).

In Kenya about 14,600 kilogram of active antimicrobials are consumed in animal production and sulfamethoxazole/trimethoprim accounts for 22 % of their total use, (Mitema *at el*., 2001). Given the fact that sulfamethoxazole/trimethoprim is the first antibiotic of choice for treating and for prophylaxis of pneumocystis jjiroveci pneumonia in HIV positive patients (Mitema et al., 2004), together with its indiscriminate use of as a result of self-medication counter, its low cost and ease accessability over and the overall exposure as an antimicrobial residue in animal products, contributes to higher resistance of *S. aureus* to sulfamethoxazole/trimethoprim (Darwish *et al*., 2013; Ondieki *et al*., 2017 and Mukokinya *et al.,* 2018), as observed in our findings.

This study shows a significant difference in overall mean resistance for *S. aureus* from human isolates to nearly all antibiotics as compared to *S. aureus* isolates from raw cattle milk. This difference can be attributed to indiscriminate use of medicines as a result of self-medication over the counter and polypharmacy. The rising cost of living in the midst of decline social-economic standards amongst the larger proportion of the population in Kenya may accelerate the vice of self-medication (Mitema *et al*., 2004). The inappropriate use of antibiotics negatively impact quality medication and treatment cost resulting to overall antimicrobial resistance amongst human patients (Mukokinya *et al.,* 2018)

Across both human and veterinary drug stores in Kenya, 28 % and 31 % of human and veterinary pharmacists respectively, considered customer preference as an important factor when prescribing an antibiotic (Muloi *et al*., 2019). In Kenya, penicillins, fluoroquinolones, and cephalosporins were the most commonly sold antibiotic classes by the human drug

retailers (Muloi *et al*., 2019), at over 50 %, 11.7% and 12.6% respectively (Mukokinya *et al.,* 2018) while in veterinary drug retailers, tetracyclines, sulfonamides, penicillins and macrolides were the most commonly sold antibiotic classes and tetracyclines and sulfonamides in poultry farmers (Muloi *et al*., 2019). The prevalence of prescribing antibiotic to human was 54.7%, in treatment units in which (75.4%) was for treatment and (29.0%) for prophylaxis. Of these, penicillins (46.9%) are the commonly prescribed, followed by cephalosporins (44.7%). Treatment guideline compliance was 45.8% (Momanyi *et al*., 2019).

Multidrug resistance *S. aureus* isolates were considered resistant to at least one antimicrobial agent in three or more antimicrobial categories according to European Centre for Disease Prevention and Control (ECDC), (2009) guidelines and Magiorakos *et al.,* (2012). In the current work, none of the isolates of *S. aureus* from raw milk showed MDR. All the 11 (42.3%) isolates were resistant to ≤ 2 different categories of anti-microbial agents therefore failed to meet the MDR definition criteria. Our data agree with finding reported in China by Dai *et al.,* (2019), who noted 2 (25%) *S. aureus* isolates free of *mec*A/*mec*C gene and only resistant to ≤ 2 antibiotics category. Contrary to our finding, a large proportion of isolates of *S. aureus* from milk reported previously from many countries exhibited multidrug 13.6 % resistance; in Ethiopian (Kalayu *et al.,* 2020), China, 6.3% (Wang *et al*., 2018), in Tanzania 26.1% (Mohammed *et al*., 2018), in Iran, 15.4% (Jamali *et al.,* 2015), in Egypt, 83 % (Awad *et al*., 2017), in Bangladesh, 49% (Hoque *et al*., (2018), 20-25% Jahan *et al.,* (2015) and in Algeria 83.9% (Matallah *et al.,* 2019). Seven resistance phenotypes were evident; TET (7.69 %), FOX-GENT (3.85 %), AMP-TET (3.84 %), ERY- SXT (3.84 %), AMP (11.54 %), FOX (7.69 %), and GENT-TET (3.85 %) wherein AMP, FOX and TET were the top three frequently identified phenotypes. The high percentage of resistance of *S. aureus* to ampicillin and

tetracycline could be due to broad administration of these antibiotics in veterinary medicine to treat or control farm related dairy infections (Jamali *et al.,* 2015).

There is a positive relationship between the presence of *mec*A gene in *S. aureus* isolate and display of multidrug resistance phenomenon (Awad *et al.,* 2017), with therefore lack of MDR among the *S. aureus* isolates from raw milk exhibited in this study could be due to *mec*A/*mec*C non expression or negativity. Also the ability of *S. aureus* strains to demonstrate wide spectra of antimicrobial resistance is positively correlated to production of β-lactamases and PBP2a (Awad *et al.,* 2017). The discrepancy in MDR observed in this study from the findings reported by many authors may be due to the fact that many studies carried out their investigations purely in animals with clinical or sub-clinical mastitis; however, in this study milk was from healthy animals.

Multidrug resistance was observed among 19 (35.84 %) isolates of *S. aureus* from human blood. Multidrug resistance to 8, 7, 6, 5 and 3 different antimicrobial combinations were determined in 4 (7.55 %), 1 (1.88%), 9 (16.98%), 1 (1.88%) and 4 (7.55%) isolates respectively. The predominant MDR phenotypes for *S. aureus* determined in this study were; FOX-AMP-CIP-GENT-AMC-SXT and FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT in 8(15.09%) and 4(7.55%) of isolates respectively (Table 4.6). In total, 9 (16.98%) MDR phenotypes were determined; FOX-AMP-CIP-ERY-GENT-TET-AMC-SXT (7.55%), FOX-AMP-CIP-ERY-GENT-AMC-SXT (1.88%), FOX-AMP-CIP-GENT-AMC-SXT (15.09%), FOX-AMP-CIP-ERY-GENT-AMC (1.88%), FOX-AMP-CIP-GENT-AMC (1.88%), FOX-AMP-SXT (1.88%), AMP-TET-SXT (1.88%), AMP-ERY-SXT (1.88%) and ERY-TET-SXT (1.88 %). The results were comparable with the findings reported by Obajuluwa *et al*., (2016). The rising occurrence of MDR-*S. aureus* isolated worldwide have been reported to harbor

multiple resistant trait that negatively affect animal and human health on the management of staphylococcal infection (Ateba *et al*.,2010). Multiple antimicrobial resistance among isolates of from cow milk and human clinical isolates may be due to acquisition of resistance factor (R-Factor) which is plasmid mediated and *S. aureus* is known to harbour a number of multiple antibiotic resistant plasmids that may explain the phenotypes observed in this study (Akindolire *et al*., 2015). Also high MDR prevalence noted in this study could be linked to either selective pressure on antimicrobial usage, increased irrational usage of antibiotics, inter-transmission of resistant genes between people, self-medication of human patients due to availability of low cost antibiotics coupled with weak legislative policies on the overall prescription of antibiotics, insufficient implementation of standard prophylactic hygienic measures and lack of infection control programs within health systems.

According to WHO (2014) report on antimicrobial resistance, the prevalence of MRSA in many African nations is poorly reported (Wangai *et al*., 2019). Overall MRSA prevalence among confirmed *S.aureus* isolates from raw cow milk was 11.54%. This finding of MRSA prevalence was consistent with Ombui *et al*., 2000 (15.6 %), Jamali *et al*., 2015, (16.2%) and Herrera *et al.,* 2016, (18.5 %) but higher than that repoted by Giacinti *et al*., (2017) of 4.4 % among dairy sheep farms in Italy. The prevalence was also lower compared to that reported in Uganda, 56.1% (Asiimwe *et al*., 2017). The discrepancy in MRSA prevalence in milk samples may be related to health status of the animals, contamination level during sample collection and poor hygienic practices in handling samples at various stages in sample processing. In the current study, the prevalence of MRSA from human blood was 37.74 %. This observation is consistent with 36 % reported in India, (Kumar *et al.,* 2017), 34.6 % in Cameroon, (Gonsu *et al*., 2013), 31.5 % in Uganda, (Ojulong *et al*., 2009). However the observations are higher than

previously reported in Nigeria of 19.2% (Olowe *et al*., 2013), in Kenya of 3.7 %, 6.9 % and 27% (Omuse *et al*., 2014; Aiken *et al*., 2014; Gitau *et al*., 2018) respectively. On the other hand, prevalence was lower than the findings in Ethiopia of 44.1 % (Shibabaw *et al*., 2013), in Sudan of 69.4% (Elimam *et al*., 2014), in Congo of 60% (Iyamba *et al*., 2014) and in Kenya of 53.4 % (Wangai *et al*., 2019).

The remarkable differences in prevalence of MRSA both in raw milk and human blood between this study and the previous intra- and inter-country need to be investigated further however factors like study design, study population and detection method, may contribute (Garoy *et al*., 2019). Studies that entirely rely on genotypic determination of MRSA by PCR reports comparably lower prevalences of MRSA (Garoy *et al*., 2019).

The gold standard to detect the genotypic information on MRSA distribution is to determine the genes conserved within staphylococcal cassette chromosome (SCC*mec*) that encodes mutant PBP2a or PBP2 (Nasution *et al*., 2018). Therefore genotypic determination of *mec*A or *mec*C is deemed as standard of reference for detection of MRSA (Stegger *et al*., 2012). All the three strains of *S. aureus* isolates from raw milk, that phenotypically expressed methicillin resistance, showed negative results for *mec*A and *mec*C following PCR assay. This finding is consistent with similar studies reported by Siripornmongcolchai *et al.,* (2002), Cekovska *et al*., (2005), Davoodi *et al*., (2012) and Pournajaf *et al*., (2014). This could be explained that the three isolates, might posses other *mec*A homologue or other mechanisms leading to β-lactam resistance (García-Álvarez *et al*., 2011). Phenotypically resistant isolates for cefoxitin/oxacillin that test negative for *mec*A by PCR, are often categorized as borderline oxacillin resistant (BORSA) or more rarely moderately resistant *S. aureus* (MODSA) strains (Stegger *et al*., 2012). Also, it's worthy to note that phenotypic expression of methicillin resistance is

dependent of medium PH, temperature and sodium chloride concentration in the medium and therefore these extra-chromosomal factors affects the agreement between harbouring of *mec*A or *mec*C gene and the expression of MRSA as evaluated by old conventional methods (Siripornmongcolchai *et al*., 2002).

However, of the 20 *S. aureus* isolates from human blood that were phenotypically resistant to cefoxitin, only 17 (85 %) isolates contained *mec*A gene. All the 3 (15 %) isolates that were *mecA* negative were also negative for *mecC* by PCR method of determination. This small proportion of isolates that showed negative amplification of the *mec*A gene and *mecC* as compared to phenotypic resistance to cefoxitin concurs with the findings reported from Egypt (Rania *et al*., 2017). Absence of *mec*A gene in phenotypically resistant *S. aureus* isolates has been reported globally (Olayinka *et al*., 2010; Elhassan *et al*., 2015; García-Garrote *et al*., 2014) that MRSA strains that didn't posses *mec*A gene, then methicillin resistance observed was due to the expression of *mec*C gene, but Cikman *et al*., (2019), reported of *S. aureus* isolates testing negative both for *mec*A and *mec*C despite being MRSA positive by phenotypic methods. A study by Becker *et al*., (2018) reported plasmid harboring mec*B* gene in the course regular diagnostic screening for MRSA in *S. aureus* isolates that showed negative test for *mec*A and *mec*C. A study for mec*B* DNA in *S. aureus*, unveiled 100 % sequence similarity with *Macroccocus caseolyticus mec*B gene therefore linked to common allotype (Lakhundi and Zhang, 2018). The native *mec*A gene from *S. aureus* N315 shows 60 % nucleotide sequence similarity to its homologue *mec*B and has shown to confer resistance to methicillin therefore known as MRSA (Lakhundi and Zhang . 2018).

The discovery of *mec*A and *mec*C genes has become a noble evidence for determination of

MRSA and is now put into practice in many countries including Kenya (Omuse *et al*., 2014, Aiken *et al*., 2014, Njoroge, (2016) and Wangai *et al*., 2019), Sudan, (Elimam *et al*., 2014, Elhassan *et al*., 2015), India, (Mehndiratta *et al*., 2009), USA, (Murakami *et al*., 1991), England, (Wongwanich *et al*., 2000), Australia, (Cloney *et al*., 1999) and in Turkey (Cikman *et al*., 2019).

MRSA strains harboring *mec*C gene may become problematic in genotypic determination (Cikman *et al*., 2019). Failure to detect *mec*A gene in resistant *S. aureus* isolate has been documented globally (Elhassan *et al*., 2015) and requires investigating alternative genetic possibilities responsible for the observed resistance (Elhassan *et al*., 2015)

The observed discrepancy between the genotypic and phenotypic observations in this study may be related to heterogeneous resistance shown by many clinical isolates and failure of expression of *mec*A or *mec*C genes. Resistance among MRSA isolates is dependent on the ability of *S. aureus* to produce PBP2a, which is equally influenced by several chromosomal and extra-chromosomal factors (Mohammed *et al*., 2018) or probability of hyper production of β- lactamase enzymes (Mohammed *et al*., 2018).

5.2. Conclusion from this study

The following conclusions were made from this study based on the data:

i. Low to moderate proportion of *S. aureus* is presnt in raw milk and human blood
ii. The presence of *S. aureus* in raw milk and human blood represents danger in dairy industry and human health
iii. The presence of MRSA in raw milk and human blood signifies the presence of notorious pathogenic strain with resistance to a wide range of antimicrobials

commonly used to combat the pathogen which may pose therapeutic challenges in veterinary or human medicine

iv. MRSA phenotypes from human blood were more resistant to various antimicrobial classes than the MRSA phenotypes isolated from raw cattle milk

v. The presence of multidrug drug resistance observed in this study signifies overuse of antimicrobials which may include self-medication and or under-use in the country

vi. Ciprofloxacin, amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole were the most effective agents against *S. aureus* isolates from raw dairy milk while ciprofloxacin, erythromycin and amoxicillin/clavulanic acid were effective against *S. aureus* from human blood

vii. Low to moderate phenotypic MRSA was observed in raw milk and human blood

viii. *S. aureus* isolates from humans were more resistant to various antimicrobial agents as compared to isolates from raw milk.

ix. All MRSA strains from raw milk did not express both *mec*A and *mec*C genes on PCR assay

x. Most of the MRSA positive *S. aureus* from human blood harboured *mec*A genes and none contained *mec*C gene

xi. The *mec*A gene in human blood is similar to some MRSA strains from animals in other parts of the world suggesting the zoonotic potential of this resistant gene

xii. About a third of *S.aureus* isolates from human blood showed MDR to various antibiotics

xiii. *S. aureus* from raw dairy milk did not show MDR

5.3. Recommendations from this study

i. Surveillance should be conducted routinely to monitor the presence of MRSA to avert the threat of increasing antimicrobial resistance

ii. The Kenyan government and the East African region to put more emphasis and formulate appropriate national and local policies and treatment guidelines both in veterinary and human medicine to tackle issues relating to MRSA and more prudently put these into effective practice to reduce the medical burden caused by this pathogen.

iii. An extensive study should be undertaken on isolates from the present work to establish the genetic relationship of resistant genes between *S. aureus* isolates from raw milk and human blood to establish the zoonotic nature of MRSA isolates from animals to humans and vice versa

REFFERENCES

Abdulgader, S. M., Shittu, A. O., Nicol, M. P., & Kaba, M. (2015). Molecular epidemiology of Methicillin-resistant Staphylococcus aureus in Africa: a systematic review. Frontiers in microbiology, 6, 348. https://doi.org/10.3389/fmicb.2015.00348

Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K. (2016). Bovine mastitis: prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa milk shed, South Ethiopia. BMC veterinary research, 12(1), 270. https://doi.org/10.1186/s12917-016-0905-3

Adhikari, R., Pant, N. D., Neupane, S., Neupane, M., Bhattarai, R., Bhatta, S., Chaudhary, R., & Lekhak, B. (2017). Detection of Methicillin Resistant Staphylococcus aureus and Determination of Minimum Inhibitory Concentration of Vancomycin for Staphylococcus aureus isolated from Pus/Wound Swab Samples of the Patients Attending a Tertiary Care Hospital in Kathmandu, Nepal. The Canadian journal of infectious diseases & medical microbiology. https://doi.org/10.1155/2017/2191532

Aiken, A. M., Mutuku, I. M., Sabat, A. J., Akkerboom, V., Mwangi, J., Scott, J. A., Morpeth, S. C., Friedrich, A. W., & Grundmann, H. (2014). Carriage of Staphylococcus aureus in Thika Level 5 Hospital, Kenya: a cross-sectional study. Antimicrobial resistance and infection control, 3, 22. https://doi.org/10.1186/2047-2994-3-22

Aires de Sousa, M., and de Lencastre, H. (2004). Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant Staphylococcus aureus clones. FEMS immunology and medical microbiology, 40(2), 101–111. https://doi.org/10.1016/S0928-8244(03)00370-5

Akindolire, M. A., Babalola, O. O., & Ateba, C. N. (2015). Detection of antibiotic resistant Staphylococcus aureus from milk: A public health implication. *International Journal*

of Environmental Research and Public Health, *12*(9), 10254–10275. https://doi.org/10.3390/ijerph120910254

Ansari, S., Nepal, H. P., Gautam, R., Rayamajhi, N., Shrestha, S., Upadhyay, G., Acharya, A., & Chapagain, M. L. (2014). Threat of drug resistant Staphylococcus aureus to health in Nepal. BMC infectious diseases, 14, 157. https://doi.org/10.1186/1471-2334-14-157

Arabzadeh F, Aeini F, Keshavarzi F, Behrvash S (2018). Resistance to Tetracycline and Vancomycin of *Staphylococcus aureus* Isolates from Sanandaj Patients by Molecular Genotyping. Ann Clin Lab Res Vol.6 No.4:260.

Arciszewska, L. K., Drake, D., & Craig, N. L. (1989). Transposon Tn7. cis-Acting sequences in transposition and transposition immunity. Journal of molecular biology, 207(1), 35–52. https://doi.org/10.1016/0022-2836(89)90439-7

Asiimwe, B. B., Baldan, R., Trovato, A., & Cirillo, D. M. (2017). Prevalence and molecular characteristics of Staphylococcus aureus, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. BMC Infectious Diseases, 17(1), 422. https://doi.org/10.1186/s12879-017-2524-4

Ateba, C. N., Mbewe, M., Moneoang, M. S., & Bezuidenhout, C. C. (2010). Antibiotic-resistant Staphylococcus aureus isolated from milk in the Mafikeng Area, North West province, South Africa. South African Journal of Science, 106(11–12), 1–6. https://doi.org/10.4102/sajs.v106i11/12.243

Awad, A., Ramadan, H., Nasr, S., Ateya, A., & Atwa, S. (2017). Genetic Characterization, Antimicrobial Resistance Patterns and Virulence Determinants of Staphylococcus aureus Isolated form Bovine Mastitis. Pakistan journal of biological sciences: PJBS, 20(6), 298–305. https://doi.org/10.3923/pjbs.2017.298.305

Ba, X., Harrison, E. M., Edwards, G. F., Holden, M. T., Larsen, A. R., Petersen, A., Skov, R. L., Peacock, S. J., Parkhill, J., Paterson, G. K., & Holmes, M. A. (2014). Novel mutations in penicillin-binding protein genes in clinical Staphylococcus aureus isolates that are methicillin resistant on susceptibility testing, but lack the mec gene. The Journal of antimicrobial chemotherapy, 69(3), 594–597. https://doi.org/10.1093/jac/dkt418

Back, S. H., Eom, H. S., Lee, H. H., Lee, G. Y., Park, K. T., & Yang, S. J. (2020). Livestock-associated methicillin-resistant Staphylococcus aureus in Korea: antimicrobial resistance and molecular characteristics of LA-MRSA strains isolated from pigs, pig farmers, and farm environment. Journal of veterinary science, 21(1), e2. https://doi.org/10.4142/jvs.2020.21.e2

Balasubramanian, D., Harper, L., Shopsin, B., & Torres, V. J. (2017). Staphylococcus aureus pathogenesis in diverse host environments. *Pathogens and disease*, *75*(1), ftx005. https://doi.org/10.1093/femspd/ftx005

Ballhausen, B., Kriegeskorte, A., Schleimer, N., Peters, G., & Becker, K. (2014). The mecA homolog mecC confers resistance against β-lactams in Staphylococcus aureus irrespective of the genetic strain background. *Antimicrobial agents and chemotherapy*, *58*(7), 3791–3798. https://doi.org/10.1128/AAC.02731-13

Bamberger, D. M., & Boyd, S. E. (2005). Management of Staphylococcus aureus infections. American family physician, 72(12), 2474–2481.

Bannerman, T.L. (2003) Staphylococcus, Micrococcus, and Other Catalase-Positive Cocci That Grow Aerobically. In: Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A.

and Yolken, R.H., Eds., Manual of Clinical Microbiology, 8th Edition, American Society for Microbiology, Washington DC, 384-404.

Basak S, Singh P, and Rajurkar M.(2015). Multidrug Resistant and Extensively Drug Resistant Bacteria:A Study. Journal of Pathogens; http://dx.doi.org/10.1155/2016/4065603

Becker, K., van Alen, S., Idelevich, E. A., Schleimer, N., Seggewiß, J., Mellmann, A., Kaspar, U., & Peters, G. (2018). Plasmid-Encoded Transferable mecB-Mediated Methicillin Resistance in Staphylococcus aureus. Emerging infectious diseases, 24(2), 242–248. https://doi.org/10.3201/eid2402.171074

Begum, R., Towhid, S. T., Moniruzzaman, M., Mia, Z., & Islam, M. A. (2011). Study of Staphylococcus aureus from clinical samples in Savar, Bangladesh. *Research Journal of Microbiology*, *6*(12), 884.

Bhatt C.P., Karki B.M.S., Baral, B., Gautam, S., Shah, A., Chaudhary, A., (2014). Antibiotic susceptibility pattern of staphylococcus aureus and methicillin-resistant staphylococcus aureus in a tertiary care hospital. Journal of Pathology of Nepal,Vol. 4, 548 - 551

Bien, J., Sokolova, O., & Bozko, P. (2011). Characterization of Virulence Factors of Staphylococcus aureus: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. Journal of pathogens, 2011, 601905. https://doi.org/10.4061/2011/601905

Bierowiec K., Korzeniowska-Kowal A., Wzorek A., Rypuła K, A. Gamian A.(2019). "Prevalence of Staphylococcus Species Colonization in Healthy and Sick Cats", BioMed Research International,: doi.org/10.1155/2019/4360525

Bonesso, M. F., Marques, S.A., Cunha, M.L.R.S., (2011). Community-acquired methicillin-

resistant Staphylococcus aureus (CAMRSA): molecular background, virulence, and relevance for public health. The Journal of Venomous Animals and Toxins including Tropical Diseases ISSN 1678-9199 | volume 17 | issue 4 | pages 378-386

Borg, M. A., Zarb, P., Scicluna, E. A., Rasslan, O., Gür, D., Ben Redjeb, S., … Daoud, Z. (2010). Antibiotic consumption as a driver for resistance in Staphylococcus aureus and Escherichia coli within a developing region. American Journal of Infection Control, 38(3), 212–216. https://doi.org/10.1016/j.ajic.2009.07.010

Boswihi, S. S. and Udo, E. E. (2018). Methicillin-resistant Staphylococcus aureus: An update on the epidemiology, treatment options and infection control. Current Medicine Research and Practice. Review Article 18–24

Boucher, H., Miller, L. and Razonable, R., (2010). Serious infections caused by methicillin-resistant Staphylococcus aureus. Journal of Clinical and Infectious Diseases, pp.183–197.

Brown, D. F., Edwards, D. I., Hawkey, P. M., Morrison, D., Ridgway, G. L., Towner, K. J., Wren, M. W., Joint Working Party of the British Society for antimicrobial chemotherapy, Hospital Infection Society, & Infection Control Nurses Association (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). The Journal of antimicrobial chemotherapy, 56(6), 1000–1018. https://doi.org/10.1093/jac/dki372.

Bush, K., & Jacoby, G. A. (2010). Updated functional classification of beta-lactamases. Antimicrobial agents and chemotherapy, 54(3),969–976. https://doi.org/10.1128/AAC.01009-09

Byarugaba, D. K. (2010). Mechanismsof Antimicrobial Resistance. In: De J. Sosa, A., Byarugaba, D. K., Amabile-Cuevas, C. F., Hsueh, P. R., Kariuki, S., & Okeke, I. N.

editors. A view on Antimicrobial Resistance in Developing Countries. ISBN 978-0-387-89369-3 Springer New York Dordrecht Heidelberg London. DOI 10.1007/978-0-387-89370-9.

CABI, (2020). Invasive Species Compendium. Wallingford, UK: CAB International. www.cabi.org/isc.

Carvalho, Karinne Spirandelli, Mamizuka, Elsa Masae, & Gontijo Filho, Paulo P. (2010). Methicillin/Oxacillin-resistant Staphylococcus aureus as a hospital and public health threat in Brazil. Brazilian Journal of Infectious Diseases, 14(1), 71-76. https://doi.org/10.1590/S1413-86702010000100014

Cekovska, Z., Panovski, N., & Petrovska, M. (2005). Methicillin-resistant Staphylococcus aureus: comparison of susceptibility test methods with mecA gene analysis for determining oxacillin (methicillin) resistance in our clinical isolates. Bratislavske Lekarske Listy, 106(4–5), 163—167. Retrieved from http://europepmc.org/abstract/MED/16080361

Cesur, S., & Demiröz, A. P. (2013). Antibiotics and the Mechanisms of Resistance to Antibiotics. Medical Journal of Islamic World Academy of Sciences, 21(4), 138–142. https://doi.org/10.12816/0002645

Chambers H. F. (2001). The changing epidemiology of Staphylococcus aureus?. Emerging infectious diseases, 7(2), 178–182. https://doi.org/10.3201/eid0702.010204

Chambers, H. F. (2005). Community-associated MRSA-resistance and virulence converge, N. Engl. J. Med. 352: 1485-1487

Chambers, H. F., & Deleo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature reviews. Microbiology, 7(9), 629–641. https://doi.org/10.1038/nrmicro2200

Chatur, Y. A., Brahmbhatt, M. N., Modi, S., & Nayak, J. B. (2014). Fluoroquinolone resistance and detection of topoisomerase gene mutation in Campylobacter jejuni isolated from animal and human sources. *Int.J.Curr.Microbiol.App.Sci*, *3*(6), 773–783.

Cheesbrough, M.,(2006). District Laboratory Practice in Tropical Countries: Part one (Second edition). Cambridge University Press, U.K. pp 143-157

Chen, C. J., & Huang, Y. C. (2014). New epidemiology of Staphylococcus aureus infection in Asia. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 20(7), 605–623. https://doi.org/10.1111/1469-0691.12705

Chon J, Sung K, Khan S.(2017). Methicillin-resistant Staphylococcus aureus (MRSA) in food-producing and companion animals and food products. Frontiers in Staphylococcus Aureus. ;8:47. doi.10.5772/66645

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews : MMBR, 65(2), 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Cikman, A., Aydin, M., Gulhan, B., Karakecili, F., Kurtoglu, M. G., Yuksekkaya, S., Parlak, M., Gultepe, B. S., Cicek, A. C., Bilman, F. B., Ciftci, I. H., Kara, M., Atmaca, S., & Ozekinci, T. (2019). Absence of the mecC gene in methicillin-resistant Staphylococcus aureus isolated from various clinical samples: The first multi-centered study in Turkey. Journal of infection and public health, 12(4), 528–533. https://doi.org/10.1016/j.jiph.2019.01.063

Cloney L, Marlowe C, Wong A, Chow R,and Bryan R, (1999). Rapid detection of mecA in methicillin resistant Saphylococcus aureus using cycling probe technology," Molecular and Cellular Probes, vol. 13, no. 3, pp. 191–197

CLSI, (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement, M100S. Wayne, P.A., 950 West Valley Road, Suite 2500.USA. PP. 56-63

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nübel, U., Ohlsen, K., Strommenger, B., Walther, B., Wieler, L., & Witte, W. (2010). Emergence of methicillin-resistant Staphylococcus aureus (MRSA) in different animal species. International journal of medical microbiology :IJMM, 300(2-3), 109–117. https://doi.org/10.1016/j.ijmm.2009.11.002

Cuny, C., Wieler, L. H., & Witte, W. (2015). Livestock-Associated MRSA: The Impact on Humans. Antibiotics (Basel, Switzerland), 4(4), 521–543. https://doi.org/10.3390/antibiotics4040521

D'amico, D. J., & Donnelly, C. W. (2011). Characterization of Staphylococcus aureus strains isolated from raw milk utilized in small-scale artisan cheese production. Journal of food protection, 74(8), 1353–1358. https://doi.org/10.4315/0362-028X.JFP-10-533

Dadashi, M., Nasiri, M. J., Fallah, F., Owlia, P., Hajikhani, B., Emaneini, M., & Mirpour, M. (2018). Methicillin-resistant Staphylococcus aureus (MRSA) in Iran: A systematic review and meta-analysis. Journal of global antimicrobial resistance, 12, 96–103. https://doi.org/10.1016/j.jgar.2017.09.006

Dai, J., Wu, S., Huang, J., Wu, Q., Zhang, F., Zhang, J., Wang, J., Ding, Y., Zhang, S., Yang, X., Lei, T., Xue, L., & Wu, H. (2019). Prevalence and Characterization of Staphylococcus aureus Isolated From Pasteurized Milk in China. Frontiers in microbiology, 10, 641. https://doi.org/10.3389/fmicb.2019.00641

Daka, D., G/Silassie, S., & Yihdego, D. (2012). Antibiotic-resistance Staphylococcus aureus isolated from cow's milk in the Hawassa area, South Ethiopia. Annals of clinical microbiology and antimicrobials, 11, 26. https://doi.org/10.1186/1476-0711-11-26

Darwish, Wageh Sobhy; Eldaly, Elsaid A.; El-Abbasy, Mohamed Tharwat; Ikenaka, Yoshinori; Nakayama, Shouta; Ishizuka, Mayumi. (2013). Antibiotic residues in food;the African scenario. Japanese J. of Veterinary Research 61 (Supplement): S13-S22

David, M. Z., & Daum, R. S. (2010). Community-Associated Methicillin-Resistant;Staphylococcus aureus: Epidemiology and Clinical Consequences of an Emerging Epidemic.Clinical Microbiology Reviews, 23(3), 616 LP – 687. https://doi.org/10.1128/CMR.00081-09

David, M. Z., Glikman, D., Crawford, S. E., Peng, J., King, K. J., Hostetler, M. A., … Daum, R. S. (2008). What is community-associated methicillin-resistant Staphylococcus aureus? Journal of Infectious Diseases, 197(9), 1235–1243. https://doi.org/10.1086/533502

Davoodi, N.R., Yousef, J.V., Harzandi, N., Hajrafi, A., Rajaei, B., Nejad, S.G., Aghasadeghi, M.R., Moshiri, A., Bahremand, A.R. and Siadat, S.D. (2012) Molecular detection of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase-negative staphylococcus (CoNS) in Iran. Afr. J. Microbiol. Res., 6(16): 3716-3721.

Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., Chen, D., Bian, H., Li, Y., & Yu, G. (2015). Resistance integrons: class 1, 2 and 3 integrons. Annals of clinical microbiology and antimicrobials, 14, 45. https://doi.org/10.1186/s12941-015-0100-6

Deplano, A., Vandendriessche, S., Nonhoff, C., & Denis, O. (2014). Genetic diversity among methicillin-resistant Staphylococcus aureus isolates carrying the mecC gene in Belgium. Journal of Antimicrobial Chemotherapy, 69(6), 1457–1460. https://doi.org/10.1093/jac/dku020

Deurenberg, R. H., & Stobberingh, E. E. (2009). The molecular evolution of hospital- and community-associated methicillin-resistant Staphylococcus aureus. Current molecular medicine, 9(2), 100–115. https://doi.org/10.2174/156652409787581637

Deyno, S., Fekadu, S., & Astatkie, A. (2017). Resistance of Staphylococcus aureus to antimicrobial agents in Ethiopia: A meta-analysis. *Antimicrobial Resistance and Infection Control*, *6*(1), 1–16. https://doi.org/10.1186/s13756-017-0243-7

Diekema, D. J., Pfaller, M. A., Schmitz, F. J., Smayevsky, J., Bell, J., Jones, R. N., Beach, M., & SENTRY Partcipants Group (2001). Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 32 Suppl 2, S114–S132. https://doi.org/10.1086/320184

Dora, N., (2011). MRSA A Global Threat. MD with distinction in International Health

Doyle, M. E., Hartmann, F. A., & Lee Wong, A. C. (2012). Methicillin-resistant staphylococci: implications for our food supply?. Animal health research reviews, 13(2), 157–180. https://doi.org/10.1017/S1466252312000187

Dufour, P., Gillet, Y., Bes, M., Lina, G., Vandenesch, F., Floret, D., Etienne, J., & Richet, H. (2002). Community-acquired methicillin-resistant Staphylococcus aureus infections

in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 35(7), 819–824. https://doi.org/10.1086/342576

Dulon, M., Haamann, F., Peters, C., Schablon, A., & Nienhaus, A. (2011). MRSA prevalence in European healthcare settings: a review. BMC infectious diseases, 11, 138. https://doi.org/10.1186/1471-2334-11-138

Duquette, R.A. and Nuttall, T.J. (2004). Methicillin-resistant Staphylococcus aureus in dogs and cats: an emerging problem? Journal of Small Animal Practice 45: 591-597.

Džidić, S., Šušković, J., and Kos, B. (2008) Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. Food Technol Biotech 46:11–21

Egege, S. R., Akani, N. P., & Nwankwo, C. E. I. (2020). Detection of Methicillin-Resistant Staphylococcus aureus in Ready-to-Eat Shellfish (Corbiculid heterodont) in Bayelsa State, Nigeria. Microbiology Research Journal International, 30(3), 22–35. https://doi.org/10.9734/mrji/2020/v30i330202

Egorov, A. M., Ulyashova, M. M., & Rubtsova, M. Y. (2018). Bacterial Enzymes and Antibiotic Resistance. Acta naturae, 10(4), 33–48.

Ektik, N., Gökmen, M., & Çıbık, R. (2017). The prevalence and antibiotic resistance of methicillin-resistant Staphylococcus aureus (MRSA) in milk and dairy products in Balikesir, Turkey. Journal of the Hellenic Veterinary Medical Society, 68(4), 613-620. http:// dx.doi.org/10.12681/jhvms.16062.

Elhassan, M. M., Ozbak, H. A., Hemeg, H. A., Elmekki, M. A., & Ahmed, L. M. (2015). Absence of the mecA Gene in Methicillin Resistant Staphylococcus aureus Isolated from Different Clinical Specimens in Shendi City, Sudan. BioMed research international, 2015, 895860. https://doi.org/10.1155/2015/895860

Elimam, M., Rehan, S., El mekki, M., & El hassan, M.. (2014). Emergence of Vancomycin Resistant and Methcillin Resistant Staphylococus aureus in Patients with Different Clinical Manifestations in Khartoum State, Sudan. Journal of American Science. 10. 106-110.

Eliopoulos, G. M., & Huovinen, P. (2001). Resistance to Trimethoprim-Sulfamethoxazole. *Clinical Infectious Diseases*, *32*(11), 1608–1614. https://doi.org/10.1086/320532

Enright, M. C., Robinson, D. A., Randle, G., Feil, E. J., Grundmann, H., & Spratt, B. G. (2002). The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). Proceedings of the National Academy of Sciences of the United States of America, 99(11), 7687–7692. https://doi.org/10.1073/pnas.122108599

European Centre for Disease Prevention and Control/European Med- icines Agency.(2009). ECDC/EMEA joint technical report: the bacterial challenge: time to react.: European centre for disease prevention and control & European medicines agency, Stockholm, Sweden & London, United Kingdom.

Eyler, R. F., & Shvets, K. (2019). Clinical Pharmacology of Antibiotics. Clinical Journal of the American Society of Nephrology, 14(7), 1080 LP – 1090. https://doi.org/10.2215/CJN.08140718

Falagas, M. E., Karageorgopoulos, D. E., Leptidis, J., & Korbila, I. P. (2013). MRSA in Africa: Filling the Global Map of Antimicrobial Resistance. PLoS ONE, 8(7). https://doi.org/10.1371/journal.pone.0068024

File, T. M. (2008). Methicillin-resistant Staphylococcus aureus (MRSA): focus on community-associated MRSA. Southern African Journal of Epidemiology and Infection, 23(2), 13–15. https://doi.org/10.1080/10158782.2008.11441307

Fitzgerald, J.R. (2012). Livestock-associated Staphylococcus aureus: origin, evolution and public health threat. Trends Microbiol; 20(4): 192–8.

Fluit, A. C., Wielders, C. L., Verhoef, J., & Schmitz, F. J. (2001). Epidemiology and susceptibility of 3,051 Staphylococcus aureus isolates from 25 university hospitals participating in the European SENTRY study. Journal of clinical microbiology, 39(10), 3727–3732. https://doi.org/10.1128/JCM.39.10.3727-3732.2001

Forbes, B. A., Sahm, D. F., Weissfeld, A. S., & Bailey, W. R. 1. (2007). Bailey & Scott's diagnostic microbiology (12th ed.). St. Louis, Mo.: Elsevier Mosby company, USA

Funaki, T., Yasuhara, T., Kugawa, S., Yamazaki, Y., Sugano, E., Nagakura, Y., … Fukuchi, K. (2019). SCCmec typing of PVL-positive community-acquired Staphylococcus aureus (CA-MRSA) at a Japanese hospital. Heliyon, 5(3), e01415. https://doi.org/10.1016/j.heliyon.2019.e01415

García-Álvarez, L., Holden, M. T., Lindsay, H., Webb, C. R., Brown, D. F., Curran, M. D., Walpole, E., Brooks, K., Pickard, D. J., Teale, C., Parkhill, J., Bentley, S. D., Edwards, G. F., Girvan, E. K., Kearns, A. M., Pichon, B., Hill, R. L., Larsen, A. R., Skov, R. L., Peacock, S. J., … Holmes, M. A. (2011). Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. The Lancet. Infectious diseases, 11(8), 595–603. https://doi.org/10.1016/S1473-3099(11)70126-8

García-Garrote, F., Cercenado, E., Marín, M., Bal, M., Trincado, P., Corredoira, J., … Vindel, A. (2014). Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia. Journal of Antimicrobial Chemotherapy, 69(1), 45–50. https://doi.org/10.1093/jac/dkt327

Garneau-Tsodikova, S., & Labby, K. J. (2016). Mechanisms of Resistance to Aminoglycoside Antibiotics: Overview and Perspectives. MedChemComm, 7(1), 11–27. https://doi.org/10.1039/C5MD00344J

Garoy, E. Y., Gebreab, Y. B., Achila, O. O., Tekeste, D. G., Kesete, R., Ghirmay, R., Kiflay, R., & Tesfu, T. (2019). Methicillin-Resistant Staphylococcus aureus (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara, Eritrea. The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale, 2019, 8321834. https://doi.org/10.1155/2019/8321834

Gheorghe, I., Popa, M., & Gabriela Măruţescu, L. (2019). Molecular Features of Virulence and Resistance Mechanisms in Nosocomial and Community-Acquired Staphylococcus aureus. Staphylococcus Aureus , Ch. 5. https://doi.org/10.5772/intechopen.75191

Giacinti, G., Carfora, V., Caprioli, A., Sagrafoli, D., Marri, N., Giangolini, G., Amoruso, R., Iurescia, M., Stravino, F., Dottarelli, S., Feltrin, F., Franco, A., Amatiste, S., & Battisti, A. (2017). Prevalence and characterization of methicillin-resistant Staphylococcus aureus carrying mecA or mecC and methicillin-susceptible Staphylococcus aureus in dairy sheep farms in central Italy. Journal of dairy science, 100(10), 7857–7863. https://doi.org/10.3168/jds.2017-12940

Gillings, M. R. (2014). Integrons: Past, Present, and Future. Microbiology and Molecular Biology Reviews, 78(2), 257 LP – 277. https://doi.org/10.1128/MMBR.00056-13

Gitau, W., Masika, M., Musyoki, M., Museve, B., & Mutwiri, T. (2018). Antimicrobial susceptibility pattern of Staphylococcus aureus isolates from clinical specimens at Kenyatta National Hospital. BMC research notes, 11(1), 226. https://doi.org/10.1186/s13104-018-3337-2

Gnanamani, A.(2017). Staphylococcus aureus: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach. In Frontiers in Staphylococcus aureus; Enany, S., Crotty Alexander, L., Eds.; IntechOpen Limited: London, UK.

Gonsu, K.H., Kouemo, S.L., Toukam, M., Ndze, V.N., Koulla, S.S., (2013). Nasal carriage of methicillin resistant Staphylococcus aureus and its antibiotic susceptibility pattern in adult hospitalized patients and medical staff in some hospitals in Cameroon. J. Microbiol. Antimicrob. 5(3):29-33. http://dx.doi. org/10.5897/JMA2012.0232

Graveland, H., Wagenaar, J. A., Bergs, K., Heesterbeek, H., & Heederik, D. (2011). Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PloS one, 6(2), e16830. https://doi.org/10.1371/journal.pone.0016830

Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E (2006). Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public- health threat. Lancet. 368(9538): 874-885. http:// dx.doi.org/10.1016/S0140-6736(06)68853-3

Grundmann, H., Aanensen, D. M., van den Wijngaard, C. C., Spratt, B. G., Harmsen, D., Friedrich, A. W., & European Staphylococcal Reference Laboratory Working Group (2010). Geographic distribution of Staphylococcus aureus causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS medicine, 7(1), e1000215. https://doi.org/10.1371/journal.pmed.1000215

Gunawardena, N.D., Thevanesam, V., Kanakaratne, N., Abeysekera, D., Ekanayake, A., Perera, N., (2012). Molecular identification of methicillin resistance and virulence marker in Staphylococcus aureus. Sri Lanka J. Infect. Dis. 2, 18–29.

Guo Y., Song G., Sun M., Wang J. and Wang Y., (2020). Prevalence and Therapies of

Antibiotic-Resistance in Staphylococcus aureus. Front. Cell. Infect. Microbiol. 10:107. doi: 10.3389/fcimb.2020.00107

Gursoy NC, Ersoy Y, Gunal S, Kuzucu C.(2009). Antibiotic resistance in Staphylococcus aureus strains isolated from blood cultures. Ankem Dergisi ; 23(1): 26-29.

Hassoun, A., Linden, P. K., & Friedman, B. (2017). Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. Critical care (London, England), 21(1), 211. https://doi.org/10.1186/s13054-017-1801-3

Hawkey P. M. (2003). Mechanisms of quinolone action and microbial response. The Journal of antimicrobial chemotherapy, 51 Suppl 1, 29–35. https://doi.org/10.1093/jac/dkg207

Herrera, F. C., García-López, M. L., & Santos, J. A. (2016). Short communication: Characterization of methicillin-resistant Staphylococcus aureus isolated from raw milk fresh cheese in Colombia. Journal of dairy science, 99(10), 7872–7876. https://doi.org/10.3168/jds.2016-11322

Hiramatsu, K., Cui, L., Kuroda, M., & Ito, T. (2001). The emergence and evolution of methicillin-resistant Staphylococcus aureus. Trends in microbiology, 9(10), 486–493. https://doi.org/10.1016/s0966-842x(01)02175-8

Hiramatsu, K., Ito, T., Tsubakishita, S., Sasaki, T., Takeuchi, F., Morimoto, Y., Katayama, Y., Matsuo, M., Kuwahara-Arai, K., Hishinuma, T., & Baba, T. (2013). Genomic Basis for Methicillin Resistance in Staphylococcus aureus. Infection & chemotherapy, 45(2), 117–136. https://doi.org/10.3947/ic.2013.45.2.117

Ho, M., McDonald, L. C., Lauderdale, T. L., Yeh, L. L., Chen, P. C., & Shiau, Y. R. (1999). Surveillance of antibiotic resistance in Taiwan, 1998. Journal of microbiology, immunology, and infection, 32(4), 239–249.

Hooper D. C. (1999). Mechanisms of fluoroquinolone resistance. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy, 2(1), 38–55. https://doi.org/10.1054/drup.1998.0068

Hoque M.N, Das Z.C, Rahman A.N.M.A, Haider M.G, Islam M.A (2018). Molecular racterization of Staphylococcus aureus strains in bovine T mastitis milk in Bangladesh; International Journal of Veterinary Science and Medicine, 6; 53–60

http://www.textbookofbacteriology.com

https://medchrome.com/wp-content/uploads/2010/05/s.aureus-agar.jpg

https://universe84a.com/wp-content/uploads/2019/07/Beta-hemolytic-colony-of-*Staphylococcus-aureus*-on-Blood-agar.jpg

Huovinen, P., Sundström, L., Swedberg, G., & Sköld, O. (1995). Trimethoprim and sulfonamide resistance. Antimicrobial agents and chemotherapy, 39(2), 279–289. https://doi.org/10.1128/aac.39.2.279

Huttner, A., Bielicki, J., Clements, M. N., Frimodt-Møller, N., Muller, A. E., Paccaud, J. P., & Mouton, J. W. (2020). Oral amoxicillin and amoxicillin–clavulanic acid: properties, indications and usage. *Clinical Microbiology and Infection*, *26*(7), 871–879. https://doi.org/10.1016/j.cmi.2019.11.028

Igbinosa, E. O., Beshiru, A., Akporehe, L. U., & Ogofure, A. G. (2016). Detection of Methicillin-Resistant Staphylococci Isolated from Food Producing Animals: A Public Health Implication. Veterinary sciences, 3(3), 14. https://doi.org/10.3390/vetsci3030014

Infections. American Family Physician www.aafp.org/afp

Islam, M.A, Kabir, S. M. L. and Rahman M. T.(2017). Molecular detection and characterization of staphylococcus aureus isolated from raw milk sold in different markets of bangladesh . Bangl. J. Vet. Med. 14 (2): 277-282

Iyamba, J. M., Wambale, J. M., Lukukula, C. M., & za Balega Takaisi-Kikuni, N. (2014). High prevalence of methicillin resistant staphylococci strains isolated from surgical site infections in Kinshasa. The Pan African medical journal, 18, 322. https://doi.org/10.11604/pamj.2014.18.322.4440

Jacoby G. A. (2005). Mechanisms of resistance to quinolones. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 41 Suppl 2, S120–S126. https://doi.org/10.1086/428052

Jacoby, G. A., & Munoz-Price, L. S. (2005). The new beta-lactamases. The New England journal of medicine, 352(4), 380–391. https://doi.org/10.1056/NEJMra041359

Jahan, M., Rahman, M., Parvej, M., Chowdhury, S. M., Haque, M., Talukder, M., & Ahmed, S. (2015). Isolation and characterization of Staphylococcus aureus from raw cow milk in Bangladesh. Journal of Advanced Veterinary and Animal Research, 2(1), 49-55. Retrieved from https://www.banglajol.info/index.php/JAVAR/article/view/21266

Jamali, H., Paydar, M., Radmehr, B., Ismail, S., & Dadrasnia, A. (2015). Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from raw milk and dairy products. Food Control, 54, 383388. doi.org/10.1016/j.foodcont.2015.02.013.

Jans, C., A. Merz, S. Johler, M. Younan, S. A. Tanner, D. W. M. Kaindi, J. Wangoh, B. Bonfoh, L. Meile, and Tasara, T. (2017). East and West African milk products are reservoirs for human and livestock-associated Staphylococcus aureus. Food Microbiol. 65:64–73. https://doi.org/10.1016/j.fm.2017.01.017.

Jarvis, W. R., Jarvis, A. A., & Chinn, R. Y. (2012). National prevalence of methicillin-resistant Staphylococcus aureus in inpatients at United States health care facilities, 2010. American journal of infection control, 40(3), 194–200. https://doi.org/10.1016/j.ajic.2012.02.001

Jevons PM. "Celbenin" – resistant Staphylococci.(1961). Br Med. J.;1:124–125. PMCID: PMC1952889

Johnson A. P. (2011). Methicillin-resistant Staphylococcus aureus: the European landscape. The Journal of antimicrobial chemotherapy, 66 Suppl 4, iv43–iv48. https://doi.org/10.1093/jac/dkr076

Johnson A. P., A. Pearson and G. Duckworth. (2005). Surveillance and epidemiology of MRSA bacteraemia in the UK. J. Antimicrob. Chemother., 56(3):455-462.

Johnston, N. J., De Azavedo, J. C., Kellner, J. D., & Low, D. E. (1998). Prevalence and characterization of the mechanisms of macrolide, lincosamide, and streptogramin resistance in isolates of Streptococcus pneumoniae. Antimicrobial agents and chemotherapy, 42(9), 2425–2426. https://doi.org/10.1128/AAC.42.9.2425

Jones, T. F., Creech, C. B., Erwin, P., Baird, S. G., Woron, A. M., & Schaffner, W. (2006). Family Outbreaks of Invasive Community-Associated Methicillin-Resistant Staphylococcus aureus Infection. Clinical Infectious Diseases, 42(9), e76–e78. https://doi.org/10.1086/503265

Kalayu, A. A., Woldetsadik, D. A., Woldeamanuel, Y., Wang, S. H., Gebreyes, W. A., & Teferi, T. (2020). Burden and antimicrobial resistance of S. aureus in dairy farms in Mekelle, Northern Ethiopia. BMC veterinary research, 16(1), 20. https://doi.org/10.1186/s12917-020-2235-8

Kateete, D. P., Bwanga, F., Seni, J., Mayanja, R., Kigozi, E., Mujuni, B., … Joloba, M. L. (2019). CA-MRSA and HA-MRSA coexist in community and hospital settings in Uganda. Antimicrobial Resistance and Infection Control, 8(1), 1–9. https://doi.org/10.1186/s13756-019-0551-1

Kennedy, A. D. and DeLeo, F. R. . (2009). Epidemiology and virulence of community-associated MRSA. Clin. Microbiol. Newsl., 31:153–160.

Kenya National Bureau of Statistics (2009). Kenya National Population and Housing Census. 2009. http://www.knbs.or.ke.

Kesah, C, Redjeb S. B., Odugbemi T. O., Boye C. S.-B., Dosso M., Ndinya Achola J. O. , Koulla-Shiro S.,Benbachir M., K. Rahal K and Borg, M. (2003). Prevalence of methicillin-resistant Staphylococcus aureus in eight African hospitals and Malta.Clinical Microbiology and Infection, 153–156.

Keyvan, Erhan, Yurdakul, Ozen, Demirtas, Ahu, Yalcin, Halil, & Bilgen, Nuket. (2020). Identification of Methicillin-Resistant Staphylococcus aureus in Bulk Tank Milk. Food Science and Technology, 40(1), 150-156. Epub January 24, 2020.https://doi.org/10.1590/fst.35818

Klevens, R. M., Edwards, J. R., Tenover, F. C., McDonald, L. C., Horan, T., Gaynes, R., & System, N. N. I. S. (2006). Changes in the Epidemiology of Methicillin-Resistant Staphylococcus aureus in Intensive Care Units in US Hospitals, 1992–2003. Clinical Infectious Diseases, 42(3), 389–391. https://doi.org/10.1086/499367

Klimešová M, Manga I, Nejeschlebová L, Horáček J, Ponížil A , Vondrušková E,(2017). Occurrence of Staphylococcus aureus in cattle, sheep, goat, and pig rearing in the Czech Republic; ACTA VET. BRNO 2017, 86: 3–10; https://doi.org/10.2754/avb201786010003.

Kluytmans, J., van Belkum, A. and Verbrugh, H. (1997). Nasal carriage of Staphylococcus aureus: epideemiology, underlying mechanisms, and associated risks, Clin. Microbiol. Rev., 10: 505-520.

Köck, R., Becker, K., Cookson, B., van Gemert-Pijnen, J. E., Harbarth, S., Kluytmans, J., … Friedrich, A. W. (2010). Methicillin-resistant Staphylococcus aureus (MRSA): Burden of disease and control challenges in Europe. Eurosurveillance, 15(41), 1–9. https://doi.org/10.2807/ese.15.41.19688-en

Kot, B., Wierzchowska, K., Piechota, M., & Gruzewska, A. (2020). Antimicrobial Resistance Patterns in Methicillin-Resistant Staphylococcus aureus from Patients Hospitalized during 2015-2017 in Hospitals in Poland. Medical Principles and Practice, 29(1), 61–68. https://doi.org/10.1159/000501788

Kourtis, A. P., Hatfield, K., Baggs, J., Mu, Y., See, I., Epson, E., Nadle, J., Kainer, M. A., Dumyati, G., Petit, S., Ray, S. M., Emerging Infections Program MRSA author group, Ham, D., Capers, C., Ewing, H., Coffin, N., McDonald, L. C., Jernigan, J., & Cardo, D. (2019). Vital Signs: Epidemiology and Recent Trends in Methicillin-Resistant and in Methicillin-Susceptible Staphylococcus aureus Bloodstream Infections - United States. MMWR. Morbidity and mortality weekly report, 68(9), 214–219. https://doi.org/10.15585/mmwr.mm6809e1

Kumar, A., Kashyap, B., & Mehndiratta, A. (2017). Comparison of conventional and rapid methods for the detection of methicillin resistance in Staphylococcus aureus from blood cultures. Tropical Journal of Medical Research, 20(2), 196. https://doi.org/10.4103/tjmr.tjmr_48_16

Lakhundi, S., & Zhang, K. (2018). Methicillin-Resistant Staphylococcus

aureus: Molecular Characterization, Evolution, and Epidemiology. Clinical Microbiology Reviews, 31(4), e00020-18. https://doi.org/10.1128/CMR.00020-18

Layer, F., Ghebremedhin, B., Moder, K. A., König, W., & König, B. (2006). Comparative study using various methods for identification of Staphylococcus species in clinical specimens. Journal of Clinical Microbiology, 44(8), 2824–2830. https://doi.org/10.1128/JCM.00226-06

Leclercq, R. (2002). Mechanisms of Resistance to Macrolides and Lincosamides: Nature of the Resistance Elements and Their Clinical Implications. Clinical Infectious Diseases, 34(4), 482–492. https://doi.org/10.1086/324626

Lee, S., Choe, P. G., Song, K. H., Park, S. W., Kim, H. B., Kim, N. J., Kim, E. C., Park, W. B., & Oh, M. D. (2011). Is cefazolin inferior to nafcillin for treatment of methicillin-susceptible Staphylococcus aureus bacteremia?. Antimicrobial agents and chemotherapy, 55(11), 5122–5126. https://doi.org/10.1128/AAC.00485-11

Lemma, F., Alemayehu, H., Stringer, A., & Eguale, T. (2021). Prevalence and Antimicrobial Susceptibility Profile of Staphylococcus aureus in Milk and Traditionally Processed Dairy Products in Addis Ababa, Ethiopia. *BioMed Research International*, *2021*. https://doi.org/10.1155/2021/5576873

Lim, W. W., Wu, P., Bond, H. S., Wong, J. Y., Ni, K., Seto, W. H., Jit, M., & Cowling, B. J. (2019). Determinants of methicillin-resistant Staphylococcus aureus (MRSA) prevalence in the Asia-Pacific region: A systematic review and meta-analysis. Journal of global antimicrobial resistance, 16, 17–27. https://doi.org/10.1016/j.jgar.2018.08.014

Liu, C., Bayer, A., Cosgrove, S. E., Daum, R. S., Fridkin, S. K., Gorwitz, R. J., Kaplan, S. L., Karchmer, A. W., Levine, D. P., Murray, B. E., J Rybak, M., Talan, D. A., Chambers, H. F., & Infectious Diseases Society of America (2011). Clinical practice guidelines by the infectious diseases society of America for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 52(3), e18–e55. https://doi.org/10.1093/cid/ciq146

Liu, H., Li, S., Meng, L., Dong, L., Zhao, S., Lan, X., Wang, J., & Zheng, N. (2017). Prevalence, antimicrobial susceptibility, and molecular characterization of Staphylococcus aureus isolated from dairy herds in northern China. Journal of dairy science, 100(11), 8796–8803. https://doi.org/10.3168/jds.2017-13370

Livermore, D.,M. (2000). Antibiotic resistance in staphylococci. International Journal of Antimicrobial Agents 16: 3-10

Locatelli, C., Cremonesi, P., Caprioli, A., Carfora, V., Ianzano, A., Barberio, A., … Moroni, P. (2017). Occurrence of methicillin-resistant Staphylococcus aureus in dairy cattle herds, related swine farms, and humans in contact with herds. Journal of Dairy Science, 100(1), 608–619. https://doi.org/10.3168/jds.2016-11797

Loewen, K., Schreiber, Y., Kirlew, M., Bocking, N., & Kelly, L. (2017). Community-associated methicillin-resistant Staphylococcus aureus infection: Literature review and clinical update. Canadian family physician Medecin de famille canadien, 63(7), 512–520.

Lowy F. D. (1998). Staphylococcus aureus infections. The New England journal of medicine, 339(8), 520–532. https://doi.org/10.1056/NEJM199808203390806

Lowy, F.D. (2003). Antimicrobial resistance: the example of Staphylococcus aureus. Journal of Clinical Investigation 111, 1265-1273

Lozano, C., Gharsa, H., Ben Slama, K., Zarazaga, M., & Torres, C. (2016). Staphylococcus aureus in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African Continent. Microorganisms, 4(1), 12. https://doi.org/10.3390/microorganisms4010012

MacDougall C. and Chambers H.F. (2011). Protein Synthesis Inhibitors and Miscellaneous Antibacterial agents. In: Brunton, L. L., Chabner, B., & Knollmann, B. C. editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics.12th Edition. New York, NY: McGraw-Hill, 2011:1521–1545

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Maina, D., Omuse, G., Revathi, G., & Adam, R. D. (2016). Spectrum of Microbial Diseases and Resistance Patterns at a Private Teaching Hospital in Kenya: Implications for Clinical Practice. PloSone, 11 (1), e0147659. https://doi.org/10.1371/journal.pone.0147659

Maina, E. K., Kiiyukia, C., Wamae, C. N., Waiyaki, P. G., & Kariuki, S. (2013). Characterization of methicillin-resistant Staphylococcus aureus from skin and soft

tissue infections in patients in Nairobi, Kenya. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, 17(2), e115–e119. https://doi.org/10.1016/j.ijid.2012.09.006

Makgotho P.E (2009). Molecular characterization of methicillin-resistance Staphylococcus aureus strains. M.Sc. Thesis. University of Peretoria

Malachowa, N., & DeLeo, F. R. (2010). Mobile genetic elements of Staphylococcus aureus. Cellular and molecular life sciences : CMLS, 67(18), 3057–3071. https://doi.org/10.1007/s00018-010-0389-4

Mandomando, I., Sigaúque, B., Morais, L., Espasa, M., Vallès, X., Sacarlal, J., Macete, E., Aide, P., Quintò, L., Nhampossa, T., Machevo, S., Bassat, Q., Menéndez, C., Ruiz, J., Roca, A., & Alonso, P. L. (2010). Antimicrobial drug resistance trends of bacteremia isolates in a rural hospital in southern Mozambique. The American journal of tropical medicine and hygiene, 83(1), 152–157. https://doi.org/10.4269/ajtmh.2010.09-0578

Matallah, A. M., Bouayad, L., Boudjellaba, S., Mebkhout, F., Hamdi, T. M., & Ramdani-Bouguessa, N. (2019). Staphylococcus aureus isolated from selected dairies of Algeria: Prevalence and susceptibility to antibiotics. Veterinary world, 12(2), 205–210. https://doi.org/10.14202/vetworld.2019.205-210

Mbindyo, C. M., Gitao, G. C., Plummer, P. J., Kulohoma, B. W., Mulei, C. M., & Bett, R. (2021). Antimicrobial resistance profiles and genes of staphylococci isolated from mastitic cow's milk in Kenya. *Antibiotics*, *10*(7), 1–14. https://doi.org/10.3390/antibiotics10070772

Mbogori, C., Muigai, A., & Kariuki, S. (2013). Detection and Characterization of Methicillin 104 Resistant Staphylococcus aureus from Toilet and Classroom Door Handles in

Selected Secondary Schools in Nairobi County. Open Journal of Medical Microbiology, 3, 248–252

Mbogori, K. C., (2016). Methicillin Resistant Staphylococcus aureus from toilet and classroom door handles in selected secondary schools in Nairobi County, Kenya.Msc. Thesis. Jomo Kenyatta University of Agriculture and Technology

McDonald L. C. (2006). Trends in antimicrobial resistance in health care-associated pathogens and effect on treatment. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 42 Suppl 2, S65–S71. https://doi.org/10.1086/499404

Mehndiratta, P. L., Bhalla, P., Ahmed, A., & Sharma, Y. D. (2009). Molecular typing of methicillin-resistant Staphylococcus aureus strains by PCR-RFLP of SPA gene: a reference laboratory perspective. Indian journal of medical microbiology, 27(2), 116–122. https://doi.org/10.4103/0255-0857.45363

Mekonnen S A , Lam T. J G.M, Hoekstra J, Rutten J.V.P.M.G, Tessema T.S, Broens E.M, Riesebos A.E, Spaninks M.P and Koop G. (2018). Characterization of Staphylococcus aureus isolated from milk samples of dairy cows in small holder farms of North-Western Ethiopia. BMC Veterinary Research ;14:246.

Misic, A.M., Davis, M.F., Tyldsley, A.S. et al.(2015) The shared microbiota of humans and companion animals as evaluated from Staphylococcus carriage sites. Microbiome 3, 2. https://doi.org/10.1186/s40168-014-0052-7

Mitema, E. S. and Kikuvi, G. M. (2004). Surveillance of the overall use of antimicrobial drugs in humans over a 5-year period (1997–2001) in Kenya. Journal of Antimicrobial Chemotherapy.DOI: 10.1093/jac/dkh43

Mitema, E. S., Kikuvi, G. M., Wegener, H. C. and Stohr, K. (2001). An assessment of

antimicrobial consumption in food producing animals in Kenya. J. Vet. Pharmacol. Ther., 24(6): 385-90.

Mohammed, J., Ziwa, M. H., Hounmanou, Y. M. G., Kisanga, A., & Tuntufye, H. N. (2018). Molecular Typing and Antimicrobial Susceptibility of Methicillin-Resistant Staphylococcus aureus Isolated from Bovine Milk in Tanzania. International Journal of Microbiology, 2018, 4287431. https://doi.org/10.1155/2018/4287431

Momanyi, L., Opanga, S., Nyamu, D., Oluka, M., Kurdi, A., & Godman, B. (2019). Antibiotic Prescribing Patterns at a Leading Referral Hospital in Kenya: A Point Prevalence Survey. Journal of research in pharmacy practice, 8(3), 149–154. https://doi.org/10.4103/jrpp.JRPP_18_68

Morgan M. (2008). Methicillin-resistant Staphylococcus aureus and animals: zoonosis or humanosis?. The Journal of antimicrobial chemotherapy, 62(6), 1181–1187. https://doi.org/10.1093/jac/dkn405

Mukokinya, M., Opanga, S., Oluka, M., & Godman, B. (2018). Dispensing of Antimicrobials in Kenya: A Cross-sectional Pilot Study and Its Implications. Journal of research in pharmacy practice, 7(2), 77–82. https://doi.org/10.4103/jrpp.JRPP_17_88

Muloi, D., Fèvre, E. M., Bettridge, J., Rono, R., Ong'are, D., Hassell, J. M., Karani, M. K., Muinde, P., van Bunnik, B., Street, A., Chase-Topping, M., Pedersen, A. B., Ward, M. J., & Woolhouse, M. (2019). A cross-sectional survey of practices and knowledge among antibiotic retailers in Nairobi, Kenya. Journal of global health, 9(2), 010412. https://doi.org/10.7189/jogh.09.020412

Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. Microbiology spectrum, 4(2),10.1128/microbiolspec.VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Muñoz-López, M., & García-Pérez, J. L. (2010). DNA transposons: nature and applications in genomics. Currentgenomics, 11(2),115–128. https://doi.org/10.2174/138920210790886871

Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H., & Watanabe, S. (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. Journal of clinical microbiology, 29(10),2240–2244. https://doi.org/10.1128/JCM.29.10.2240-2244.1991

Nasution S. G, Suryanto D, and Kusumawati L.R (2018). Detection of meca gene from methicillin resistant staphylococcus aureus isolates of north sumatera. IOP Conf. Ser.: Earth Environ. Sci. 130

Ngatia, T. A. (1988). Aetiology and pathology of subclinical mastitis in dairy cows. Ph.D. Diss., Univ. Nairobi, Kenya.

Nikaido H. (2009). Multidrug resistance in bacteria. Annual review of biochemistry, 78, 119–146. https://doi.org/10.1146/annurev.biochem.78.082907.145923

Njoroge Cecilia (2016). Phenotypic and molecular characterization of methicillin resistant staphylococcus aureus from surgical patients and normal dogs, Msc.Thesis, Nairobi University

Nordmann, P., & Poirel, L. (2005). Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. The Journal of antimicrobial chemotherapy, 56(3), 463–469. https://doi.org/10.1093/jac/dki245

Obajuluwa, A., Olayinka, B., Adeshina, G., Igwe, J.C. and Onaolapo, J. (2016). Antimicrobial Susceptibility Pattern of Staphylococcus aureus Isolatesfrom Orthopaedic Patients in Abuth, Zaria. Journal of Food & Industrial Microbiology. 02. 10.4172/2572-4134.1000106.

Ojulong, J., Mwambu, T. P., Joloba, M., Bwanga, F., & Kaddu-Mulindwa, D. H. (2009). Relative prevalence of methicilline resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. Tanzania journal of health research, 11(3), 149–153. https://doi.org/10.4314/thrb.v11i3.47703

Okon, K. O., Uba, A. B. P., Oyawoye, O. M., Yusuf, I. Z., Shittu, A. O., & Blanc, D. L. J. (2011). Epidemiology and characteristic pattern of methicillin-resistant Staphylococcus aureus recovered from tertiary hospitals in Northeastern, Nigeria. International Journal of Tropical Medicine, 6(5), 106–112.

Okonkwo EC, Orji JO, Aondoackaa AD, Ugbo EN, Moses IB, et al. (2018). Prevalence and Antibiotic Sensitivity Pattern of Staphylococcus aureus Isolates of Non-Hospital Origin. Arch Clin Microbiol. Vol.9 No.1:76

Ola, S. (2001). Resistance to trimethoprim and sulfonamides. Veterinary Research, BioMed Central, 32 (3-4), pp.261-273.

Ola, S. (2010). Sulfonamides and trimethoprim. Expert Rev. Anti infects. Ther. 8(1), 1–6

Olalekan A, Schaumburg F, Nurjadi D, Dike A, Ojurongbe O, Kolawole D, et al.(2012). Clonal expansion accounts for an excess of antimicrobial resistance in Staphylococcus aureus colonising HIV-positive individuals in Lagos, Nigeria. Int J Antimicrob Agents ;40:268–72.

Olayinka, B. ., Olayinka, A. ., Obajuluwa, A. ., Onaolapo, J. ., & Olurinola, P. . (2010). Absence of mecA gene in methicillin-resistant staphylococcus aureus isolates. *African Journal of Infectious Diseases*, *3*(2), 49–56. https://doi.org/10.4314/ajid.v3i2.55081

Oliveira, D. C., and de Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant

Staphylococcus aureus. Antimicrobial agents and chemotherapy, 46(7), 2155–2161. https://doi.org/10.1128/aac.46.7.2155-2161.2002

Olowe, O. A., Kukoyi, O. O., Taiwo, S. S., Ojurongbe, O., Opaleye, O. O., Bolaji, O. S., Adegoke, A. A., Makanjuola, O. B., Ogbolu, D. O., & Alli, O. T. (2013). Phenotypic and molecular characteristics of methicillin-resistant Staphylococcus aureus isolates from Ekiti State, Nigeria. Infection and drug resistance, 6, 87–92. https://doi.org/10.2147/IDR.S48809

Ombui, J. N., Arimi, S. M., & Kayihura, M. (1992). Raw milk as a source of enterotoxigenic Staphylococcus aureus and enterotoxins in consumer milk. East African medical journal, 69(3), 123–125.

Ombui, J. N., Kimotho, A. M., & Nduhiu, J. G. (2000). Antimicrobial resistance patterns and plasmid profiles of Staphylococcus aureus isolated from milk and meat. East African medical journal, 77(9), 463–467. https://doi.org/10.4314/eamj.v77i9.46688

Omoshaba, E.O., Ojo, O.E., Oyekunle, M.A. et al., (2020). Methicillin-resistant Staphylococcus aureus (MRSA) isolated from raw milk and nasal swabs of small ruminants in Abeokuta, Nigeria. Trop Anim Health Prod 52, 2599–2608; https://doi.org/10.1007/s11250-020-02301-x

Omuse, G., Kabera, B., & Revathi, G. (2014). Low prevalence of methicillin resistant Staphylococcus aureus as determined by an automated identification system in two private hospitals in Nairobi, Kenya: a cross sectional study. BMC infectious diseases, 14, 669. https://doi.org/10.1186/s12879-014-0669-y

Omwenga, I., Aboge, G. O., Mitema, E. S., Obiero, G., Ngaywa, C., Ngwili, N., Wamwere, G., Wainaina, M., & Bett, B. (2019). Staphylococcus aureus enterotoxin genes

detected in milk from various livestock species in northern pastoral region of Kenya. Food Control, 103, 126–132. https://doi.org/10.1016/j.foodcont.2019.04.005.

Ondieki K. G, Ombui N.J.,Obonyo M.,Gura Z.,Githuku J., Orinde B.A.,and Joseph Kangangi Gikunju K.J.(2017). Antimicrobial residues and compositional quality of informally marketed raw cow milk, Lamu West Sub-County, Kenya, 2015. The Pan African Medical Journal; 28 (Supp 1): 5 . DOI: 10.11604/pamj.supp.2017.28.1.9279

Otto M. (2012). MRSA virulence and spread. Cellular microbiology, 14(10), 1513–1521. https://doi.org/10.1111/j.1462-5822.2012.01832.x

Otto M. (2014). Staphylococcus aureus toxins. Current opinion in microbiology, 17, 32–37. https://doi.org/10.1016/j.mib.2013.11.004

Pantosti, A., & Venditti, M. (2009). What is MRSA? European Respiratory Journal, 34(5), 1190 LP – 1196. https://doi.org/10.1183/09031936.00007709

Parisi, A., Caruso, M., Normanno, G., Latorre, R., Sottili, A., Miccolupo, R., Fraccalvieri, G., & Santagada, G. (2016). Prevalence, antimicrobial susceptibility and molecular typing of methicillin-resistant Staphylococcus aureus (MRSA) in bulk tank milk from southern Italy. Food Microbiology, 58, 36-42. http://dx.doi.org/10.1016/j.fm.2016.03.004. PMid:27217357

Paterson, G. K., Harrison, E. M., & Holmes, M. A. (2014). The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends in microbiology, 22(1), 42–47. https://doi.org/10.1016/j.tim.2013.11.003

Paterson, G. K., Morgan, F. J., Harrison, E. M., Peacock, S. J., Parkhill, J., Zadoks, R. N., & Holmes, M. A. (2014). Prevalence and properties of mecC methicillin-resistant Staphylococcus aureus (MRSA) in bovine bulk tank milk in Great Britain. The

Journal of antimicrobial chemotherapy, 69(3), 598–602. https://doi.org/10.1093/jac/dkt417

Patrick, Graham L.(2013). An Introduction to Medicinal Chemistry 5th Edition. New York: Oxford University Press, Pg.413-415.

Peters, L., Olson, L., Khu, D. T. K., Linnros, S., Le, N. K., Hanberger, H., … Larsson, M. (2019). Multiple antibiotic resistance as a risk factor for mortality and prolonged hospital stay: A cohort study among neonatal intensive care patients with hospital-acquired infections caused by gram-negative bacteria in Vietnam. PLoS ONE, 14(5), 1–18. https://doi.org/10.1371/journal.pone.0215666

Petersen, A., M. Stegger, O. Heltberg, J. Christensen, A. Zeuthen, L. K. Knudsen, T. Urth, M. Sorum, L. Schouls, J. Larsen, R. Skov, and A. R. Larsen. (2013). Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin. Microbiol. Infect. 19:E16–E22. https://doi.org/10.1111/1469 -0691.12036.

Petinaki, E., Arvaniti, A., Dimitracopoulos, G., & Spiliopoulou, I. (2001). Detection of mecA, mecR1 and mecI genes among clinical isolates of methicillin-resistant staphylococci by combined polymerase chain reactions. Journal of Antimicrobial Chemotherapy, 47(3), 297–304. https://doi.org/10.1093/jac/47.3.297

Petri, W. A. (2006). Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition (Laurence L. Brunton, Bruce A. Chabner, Knollmann, C. ed.), McGaw Hill Companies Inc. USA. PP. 1477- 1501.

Pourmand, M. R., Hassanzadeh, S., Mashhadi, R., & Askari, E. (2014). Comparison of four diagnostic methods for detection of methicillin resistant Staphylococcus aureus. Iranian journal of microbiology, 6(5), 341–344.

Pournajaf, A., Ardebili, A., Goudarzi, L., Khodabandeh, M., Narimani, T., & Abbaszadeh, H. (2014). PCR-based identification of methicillin-resistant Staphylococcus aureus strains and their antibiotic resistance profiles. Asian Pacific journal of tropical biomedicine, 4(Suppl 1), S293–S297. https://doi.org/10.12980/APJTB.4.2014C423

Quintiliani, R. and Courvalin, P. (1995). Mechanisms of resistance to antimicrobial agents, In Manual of Clinical Microbiology, ed P. R. Murray, E. J. Baron, M. A. Pfaller, F. R. Tenover, and R. H. Yolken, pp. 1308–1326. Washington, D.C: ASM Press.

Rahman, M. M., Amin, K. B., Rahman, S., Khair, A., Rahman, M., Hossain, A., Rahman, A., Parvez, M. S., Miura, N., & Alam, M. M. (2018). Investigation of methicillin-resistant Staphylococcus aureus among clinical isolates from humans and animals by culture methods and multiplex PCR. BMC veterinary research, 14(1), 300. https://doi.org/10.1186/s12917-018-1611-0

Rania, A. A., Nsreen, M.K., Rasha, H.E.l., Mona, M.A. (2017). Evaluation for the Novel mecC Methicillin Resistance among Methicillin Resistant Staphylococcal Isolates in two Egyptian University Hospitals. Arch Clin Microbiol. Vol.9 No.1:71

Raygada, J. L., & Levine, D. P. (2009). Methicillin-Resistant Staphylococcus aureus: A Growing Risk in the Hospital and in the Community. American health & drug benefits, 2(2), 86–95.

Reddy, P. N., Srirama, K., & Dirisala, V. R. (2017). An Update on Clinical Burden, Diagnostic Tools, and Therapeutic Options of Staphylococcus aureus. Infectious diseases, 10, 1179916117703999. https://doi.org/10.1177/1179916117703999

Rizvi, Syed. (2018). Tetracycline: Classification, Structure Activity Relationship and Mechanism of Action as a Theranostic Agent for Infectious Lesions-A Mini Review.

Biomedical Journal of Scientific & Technical Research. 7. 10.26717/BJSTR.2018.07.001475.

Ryan, K.J. and Ray, C.G., (2004). Sherris Medical Microbiology. An introduction to infectious diseases, New York: Mcgraw-Hill.

Sağlam, A. G., Şahin, M., Çelik, E., Çelebi, Ö., Akça, D., & Otlu, S. (2017). The role of staphylococci in subclinical mastitis of cows and lytic phage isolation against to Staphylococcus aureus. Veterinary world, 10(12), 1481–1485. https://doi.org/10.14202/vetworld.2017.1481-1485

Sato, T., Usui, M., Konishi, N., Kai, A., Matsui, H., Hanaki, H., & Tamura, Y. (2017). Closely related methicillin-resistant Staphylococcus aureus isolates from retail meat, cows with mastitis, and humans in Japan. PloS one, 12(10), e0187319. https://doi.org/10.1371/journal.pone.0187319

Schaumburg, F, Pauly, M, Anoh, E, Mossoun, A, Wiersma, L.C.M, Schubert, G, … Peters, G. (2015). Staphylococcus aureus complex from animals and humans in three remote African regions. Clinical Microbiology and Infection, 21(4). doi:10.1016/j.cmi.2014.12.001

Schaumburg, F., Alabi, A. S., Peters, G., & Becker, K. (2014). New epidemiology of Staphylococcus aureus infection in Africa. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 20(7), 589–596. https://doi.org/10.1111/1469-0691.12690

Sergelidis.D and Angelidis. A.S (2017). Methicillin-resistant Staphylococcus aureus: a controversial food-borne pathogen. Letters in Applied Microbiology. Review Article,. 64, 409--418

Sharma, M., Nunez-Garcia, J., Kearns, A. M., Doumith, M., Butaye, P. R., Angeles Argudín, M., … Anjum, M. F. (2016). Livestock-associated methicillin resistant Staphylococcus aureus (LA-MRSA) clonal complex (CC) 398 isolated from UK animals belong to European lineages. Frontiers in Microbiology, 7(NOV), 1–13. https://doi.org/10.3389/fmicb.2016.01741

Shi, L., Wang, H., & Lu, Z. (2016). Staphylococcal Infection and Infertility. Genital Infections and Infertility. https://doi.org/10.5772/62663

Shibabaw, A., Abebe, T., & Mihret, A. (2013). Nasal carriage rate of methicillin resistant Staphylococcus aureus among Dessie Referral Hospital Health Care Workers; Dessie, Northeast Ethiopia. Antimicrobial resistance and infection control, 2(1), 25. https://doi.org/10.1186/2047-2994-2-25

Shitandi, A., & Sternesjö, A. (2004). Prevalence of multidrug resistant Staphylococcus aureus in milk from large- and small-scale producers in Kenya. Journal of dairy science, 87(12), 4145–4149. https://doi.org/10.3168/jds.S0022-0302(04)73557-2

Siddiqui, A. H., & Koirala, J. (2021). Methicillin Resistant Staphylococcus Aureus. In StatPearls. StatPearls Publishing.

Siripornmongcolchai, T., Chomvarin, C., Chaicumpar, K., Limpaiboon, T., & Wongkhum, C. (2002). Evaluation of different primers for detecting mecA gene by PCR in comparison with phenotypic methods for discrimination of methicillin-resistant Staphylococcus aureus. The Southeast Asian journal of tropical medicine and public health, 33(4), 758–763.

Song, J.-H., Hsueh, P.-R., Chung, D. R., Ko, K. S., Kang, C.-I., Peck, K. R., … Carlos, C. C. (2011). Spread of methicillin-resistant Staphylococcus aureus between the community and

the hospitals in Asian countries: an ANSORP study. Journal of Antimicrobial Chemotherapy, 66(5), 1061–1069. https://doi.org/10.1093/jac/dkr024

Stapleton, P. D., & Taylor, P. W. (2002). Methicillin resistance in Staphylococcus aureus: mechanisms and modulation. *Science progress*, *85*(Pt 1), 57–72. https://doi.org/10.3184/003685002783238870

Stegger, M., Andersen, P. S., Kearns, A., Pichon, B., Holmes, M. A., Edwards, G., Laurent, F., Teale, C., Skov, R., & Larsen, A. R. (2012). Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 18(4), 395–400. https://doi.org/10.1111/j.1469-0691.2011.03715.x

Sudhanthiramani, S., Swetha, C. S., & Bharathy, S. (2015). Prevalence of antibiotic resistant Staphylococcus aureus from raw milk samples collected from the local vendors in the region of Tirupathi, India. Veterinary world, 8(4), 478–481. https://doi.org/10.14202/vetworld.2015.478-481

Tanwar, J., Das, S., Fatima, Z., & Hameed, S. (2014). Multidrug Resistance: An Emerging Crisis. Interdisciplinary Perspectives on Infectious Diseases, 2014, 541340. https://doi.org/10.1155/2014/541340

Taylor, D. E., & Chau, A. (1996). Tetracycline resistance mediated by ribosomal protection. Antimicrobial agents and chemotherapy, 40(1), 1–5. https://doi.org/10.1128/AAC.40.1.1

Tenover F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. The American journal of medicine, 119(6Suppl1),S3–S70.

Thesis. UT Southwestern Medical School.

Thomson C. J. (1993). Trimethoprim and brodimoprim resistance of gram-positive and gram-negative bacteria. Journal of chemotherapy (Florence, Italy), 5(6), 458–464.

Tiemersma, E. W., Bronzwaer, S. L. A. M., Lyytikäinen, O., Degener, J. E., Schrijnemakers, P., Bruinsma, N., … Whale, M. (2004). Methicillin-resistant staphylococcus aureus in Europe, 1999-2002. *Emerging Infectious Diseases*, *10*(9), 1627–1634. https://doi.org/10.3201/eid1009.040069

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews, 28(3), 603–661. https://doi.org/10.1128/CMR.00134-14

Umaru, G. ., Kwaga, J. K. ., Bello, M., Raji, M. ., & Maitala, Y. . (2016). Antibiotic resistance of Staphylococcus aureus isolated from fresh cow milk in settled Fulani herds in Kaduna State, Nigeria |. Bulletin of Animal Health and Production in Africa, 64(March), 173–182.

United Nations Commission on Human Settlements (1996). An urbanizing world: global report on human settlements.

Uwaezuoke, J. C., & Aririatu, L. E. (2005). A Survey of Antibiotic Resistant Staphylococcus Aureus Strains from Clinical Sources in Owerri. Journal of Applied Sciences and Environmental Management, 8(1). https://doi.org/10.4314/jasem.v8i1.17230

Valsesia, G., Rossi, M., Bertschy, S., & Pfyffer, G. E. (2010). Emergence of SCCmec type IV and SCCmec type V methicillin-resistant Staphylococcus aureus containing the Panton-Valentine leukocidin genes in a large academic teaching hospital in central

Switzerland: external invaders or persisting circulators?. Journal of clinical microbiology, 48(3), 720–727. https://doi.org/10.1128/JCM.01890-09

W H O. (2014). Antimicrobial resistance: global report on surveillance. Geneva: World Health Organization. https://apps.who.int/iris/handle/10665/112642

Waldvogel, FA .(2000). Staphylococcus aureus (including toxic shock syndrome). In: Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases. (5th ed). Churchill Living

Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. Nature, 406(6797), 775–781. https://doi.org/10.1038/35021219

Wang, R. F., Cao, W. W., & Cerniglia, C. E. (1997). A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. Journal of Applied Microbiology, 83(6), 727–736. https://doi.org/10.1046/j.1365-2672.1997.00300.x

Wang, W., Lin, X., Jiang, T., Peng, Z., Xu, J., Yi, L., Li, F., Fanning, S., & Baloch, Z. (2018). Prevalence and Characterization of Staphylococcus aureus Cultured From Raw Milk Taken From Dairy Cows With Mastitis in Beijing, China. Frontiers in microbiology, 9, 1123. https://doi.org/10.3389/fmicb.2018.01123

Wang, X., Li, G., Xia, X., Yang, B., Xi, M., & Meng, J. (2014). Antimicrobial susceptibility and molecular typing of methicillin-resistant staphylococcus aureus in retail foods in Shaanxi, China. Foodborne pathogens and disease, 11(4), 281–286. https://doi.org/10.1089/fpd.2013.1643

Wangai, F. K., Masika, M. M., Maritim, M. C., & Seaton, R. A. (2019). Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa: red alert or red herring? BMC Infectious Diseases, 19(1), 596. https://doi.org/10.1186/s12879-019-4245-3

Watkins, R. R., David, M. Z., & Salata, R. A. (2012). Current concepts on the virulence mechanisms of meticillin-resistant Staphylococcus aureus. Journal of medical microbiology, 61(Pt 9), 1179–1193. https://doi.org/10.1099/jmm.0.043513-0

Williams, D. J., Cooper, W. O., Kaltenbach, L. A., Dudley, J. A., Kirschke, D. L., Jones, T. F., Arbogast, P. G., Griffin, M. R., & Creech, C. B. (2011). Comparative effectiveness of antibiotic treatment strategies for pediatric skin and soft-tissue infections. Pediatrics, 128(3), e479–e487. https://doi.org/10.1542/peds.2010-3681

Wongwanich, S., Tishyadhigama, P., Paisomboon, S., Ohta, T., & Hayashi, H. (2000). Epidemiological analysis of methicillin resistant Staphylococcus aureus in Thailand. The Southeast Asian journal of tropical medicine and public health, 31(1), 72–76.

Yılmaz, E. Ş., & Aslantaş, Ö. (2017). Antimicrobial resistance and underlying mechanisms in Staphylococcus aureus isolates. *Asian Pacific Journal of Tropical Medicine*, *10*(11), 1059–1064. https://doi.org/10.1016/j.apjtm.2017.10.003

Zhang, T., Wang, C. G., Lv, J. C., Wang, R. S., & Zhong, X. H. (2012). Survey on tetracycline resistance and antibiotic-resistant genotype of avian Escherichia coli in North China. *Poultry Science*, *91*(11), 2774–2777. https://doi.org/10.3382/ps.2012-02453

Zhu, L., Zou, F., Yan, Y., Wang, Q., Shi, Y., & Qu, W. (2016). The Characteristics of Staphylococcus aureus Small Colony Variant Isolated from Chronic Mastitis at a Dairy Farm in Yunnan Province, China. The Scientific World Journal, 2016, 9157605. https://doi.org/10.1155/2016/9157605

APPENDICES

Appendix 1: Clinical and Laboratory Standards Institute (CLSI) Guideline M100, Table 2C. Zone Diameter and Minimal Inhibitory Concentration (MIC) Breakpoints for Staphylococcus spp.

Table 2C
Staphylococcus spp.
M02 and M07

56

Table 2C. Zone Diameter and Minimal Inhibitory Concentration Breakpoints for *Staphylococcus* spp.

Testing Conditions	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; CAMHB+ 2% NaCl for oxacillin; CAMHB supplemented to 50 µg/mL calcium for daptomycin Agar dilution: MHA; MHA + 2% NaCl for oxacillin. Agar dilution has not been validated for daptomycin.
Inoculum:	Direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours; 24 hours (CoNS and cefoxitin) Dilution methods: 16–20 hours; 24 hours for oxacillin and vancomycin; Testing at temperatures above 35°C may not detect MRS.

Routine QC Recommendations (See Tables 4A and 5A for acceptable QC ranges.)

Disk diffusion:
Staphylococcus aureus ATCC® 25923

Dilution methods:
Staphylococcus aureus ATCC® 29213

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02-A12, Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light, except for linezolid, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter. For linezolid, any discernible growth within the zone of inhibition is indicative of resistance to the respective agent.

(2) For staphylococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07-A10, Figures 3 and 4). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥80% reduction in growth as compared to the control (see M07-A10, Figure 2).

(3) Historically, resistance to the penicillinase-stable penicillins (see Glossary I) has been referred to as "methicillin resistance" or "oxacillin resistance." MRSAs are those strains of *S. aureus* that express *mecA* or another mechanism of methicillin resistance, such as changes in affinity of penicillin-binding proteins for oxacillin (modified *S. aureus* strains).

M100, 27th ed.

Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm) S	I	R	Interpretive Categories and MIC Breakpoints (µg/mL) S	I	R	Comments
PENICILLINASE-STABLE PENICILLINS (Continued)									
A	Oxacillin (For *S. aureus* and *S. lugdunensis*)		–	–	–	≤2 (oxacillin)	–	≥4 (oxacillin)	For use with *S. aureus* and *S. lugdunensis*. **(12)** Oxacillin disk testing is not reliable. See cefoxitin and **general** comment (5) for reporting oxacillin when testing cefoxitin as a surrogate agent.
		30 µg cefoxitin (surrogate test for oxacillin)	≥22	–	≤21	≤4 (cefoxitin)	–	≥8 (cefoxitin)	**(13)** Cefoxitin is tested as a surrogate for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result. **(14) Cefoxitin MIC and disk diffusion tests performed on media other than CAMHB or unsupplemented MHA do not reliably detect *mecA*-mediated resistance in isolates of *S. aureus* that do not grow on these media (eg, small colony variants). Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 hours incubation in 5% CO_2) or *mecA* should be done. Isolates that test either *mecA* negative or PBP2a negative or cefoxitin susceptible should be reported as oxacillin susceptible.** See **general** comment **(4)** and (5) **and comments** (8) and (11).

Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm)			Interpretive Categories and MIC Breakpoints (µg/mL)			Comments
			S	I	R	S	I	R	
AMINOGLYCOSIDES									
(24) For staphylococci that test susceptible, aminoglycosides are used only in combination with other active agents that test susceptible.									
C	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
O	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Kanamycin	30 µg	≥18	14–17	≤13	≤16	32	≥64	
O	Netilmicin	30 µg	≥15	13–14	≤12	≤8	16	≥32	
O	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
MACROLIDES									
(25) Not routinely reported on organisms isolated from the urinary tract.									
A	Azithromycin or	15 µg	≥18	14–17	≤13	≤2	4	≥8	
A	clarithromycin or	15 µg	≥18	14–17	≤13	≤2	4	≥8	
A	erythromycin	15 µg	≥23	14–22	≤13	≤0.5	1–4	≥8	
O	Telithromycin	15 µg	≥22	19–21	≤18	≤1	2	≥4	
O	Dirithromycin	15 µg	≥19	16–18	≤15	≤2	4	≥8	
TETRACYCLINES									
(26) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
B	Tetracycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
B	Doxycycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	See comment (25).
FLUOROQUINOLONES									
(27) *Staphylococcus* spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.									
C	Ciprofloxacin or	5 µg	≥21	16–20	≤15	≤1	2	≥4	
C	levofloxacin	5 µg	≥19	16–18	≤15	≤1	2	≥4	
C	Moxifloxacin	5 µg	≥24	21–23	≤20	≤0.5	1	≥2	
O	Enoxacin	10 µg	≥18	15–17	≤14	≤2	4	≥8	**(28) For testing and reporting of urinary tract isolates only.**
O	Gatifloxacin	5 µg	≥23	20–22	≤19	≤0.5	1	≥2	
O	Grepafloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Lomefloxacin	10 µg	≥22	19–21	≤18	≤2	4	≥8	
O	Norfloxacin	10 µg	≥17	13–16	≤12	≤4	8	≥16	**See comment (28).**
O	Ofloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Sparfloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
Inv.	Fleroxacin	5 µg	≥19	16–18	≤15	≤2	4	≥8	
FOLATE PATHWAY INHIBITORS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	
U	Sulfonamides	250 or 300 µg	≥17	13–16	≤12	≤256	–	≥512	**(30)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥16	11–15	≤10	≤8	–	≥16	

Appendix 2: Regional distribution of MRSA resistance to β-lactam antibiotics globally.

Data sources based on at least 30 tested isolates	Overall reported range of resistance proportion in %	Reported range of resistance proportion % in invasive isolates (No. of reports)
African region		
- National data (n = 9 countries)	12 – 80	52 (n = 1)
- from 10 additional countries	0 – 100	33 – 95 (n = 3)
Americas region		
- National data or report to ReLAVRA(n=15 countries)	21 – 90	
- National networks (n=2) no additional country .	21 – 84 24 – 90	43 – 45 (n=2)
- Publications (n = 17) from 7 additional countries		
Eastern Mediterranean region		
- National data (n = 4 countries) Hospital networks (n = 1)	10 – 53 46	53 (n = 1)
- From 1 additional publication (n=31)	0 – 92	13 – 18 (n = 3)
- From 10 additional countries		
European region		
- National data or report to EARs – Net n = 36 countries)	0.3 – 60	0.3 – 6 (n = 32)
- Publications (n=5) from 2 additional	27 – 80	27 – 50 (n = 3)

countries		
South East Asia region - National reports (n=3) countries - Publications (n = 25) from 4 additional countries	 10 – 26 2 – 80	 37 (n - 1)
Western Pacific region - National data (n = 16) countries - Institute surveillance (n = 2) from one additional country - Publication (n = 1) from one additional country	 4 – 84 1 – 4 60	

Source: Antimicrobial resistance, Global report on surveillance

WHO, 2014

EARS – Net – European antimicrobial resistance surveillance network

ReLAVRA – Latin America Antimicrobial resistance surveillance network

Appendix 3: Phenotypic characterization of Antimicrobial Resistance patterns

Panel of Antibiotics used;

Cefoxitin (FOX)
30µg
Ampicillin (AMP) 10µg
Ciprofloxacin (CIP) 5µg
Erythromycin (ERY) 15µg
Gentamycin (GENT) 10µg
Tetracycline (TET) 30µg
Amoxicillin - Calvulanic Acid
(AMC) 30µg
Sulfamethoxazole - Trimethoprim (SXT)
25µg

Antimicrobial susceptability testing for S. aureus isolates from raw cow milk

Sample No.	Diameter (mm)	FOX 30µg	AMP 10µg	CIP 5µg	ERY 15µg	GENT 10µg	TET 30µg	AMC 30µg	SXT 25µg
ATCC 25923	D1	29	27	26	24	29	26	25	30
	D2	29	25	25	24	27	24	24	28
	AVERAGE	29	26	26	24	28	25	25	29
116	D1	29	19	30	28	20	31	25	25
	D2	29	20	29	28	18	29	25	25
	AVERAGE	29	20	30	28	19	30	25	25
119	D1	36	28	35	38	30	14	35	36
	D2	34	30	34	37	21	14	36	35
	AVERAGE	35	29	35	38	26	14	36	36

120	D1	34	38	40	30	26	34	40	40
	D2	34	37	40	36	24	36	41	39
	AVERAGE	34	38	40	33	25	35	41	40
122	D1	32	22	32	35	26	10	32	32
	D2	32	22	33	35	25	10	31	34
	AVERAGE	32	22	33	35	26	10	32	33
123	D1	28	20	28	26	20	27	26	26
	D2	27	18	29	27	19	26	25	25
	AVERAGE	28	19	29	27	20	27	26	26
132	D1	22	23	28	27	10	27	34	26
	D2	20	22	27	26	9	26	31	25
	AVERAGE	21	23	28	27	10	27	33	26
137	D1	27	19	29	26	20	28	30	26
	D2	27	19	28	25	19	26	28	25
	AVERAGE	27	19	29	26	20	27	29	26
139	D1	24	27	30	30	22	29	29	33
	D2	24	26	30	29	23	28	30	31
	AVERAGE	24	27	30	30	23	29	30	32
156	D1	28	14	26	20	18	10	25	24
	D2	27	14	26	22	17	10	27	23
	AVERAGE	28	14	26	21	18	10	26	24
161	D1	26	12	26	24	24	25	24	26
	D2	26	12	25	23	23	24	25	26
	AVERAGE	26	12	26	24	24	25	25	26
162	D1	28	16	27	24	20	24	27	26
	D2	26	16	27	23	18	23	28	25
	AVERAGE	27	16	27	24	19	24	28	26
170	D1	25	35	25	24	18	23	36	13
	D2	25	36	26	25	16	25	38	12
	AVERAGE	25	36	26	25	17	24	37	13
209	D1	26	16	25	23	16	25	23	21
	D2	28	15	21	25	16	26	24	21
	AVERAGE	27	16	23	24	16	26	24	21

219	D1	30	29	27	28	20	29	36	29
	D2	29	27	30	29	19	30	32	29
	AVERAGE	30	28	29	29	20	30	34	29
221	D1	30	37	30	30	20	21	38	28
	D2	32	42	32	29	21	32	40	27
	AVERAGE	31	40	31	30	21	27	39	28
222	D1	32	35	30	27	21	28	35	26
	D2	34	39	26	28	20	29	40	26
	AVERAGE	33	37	28	28	21	29	38	26
223	D1	37	41	30	32	22	33	40	30
	D2	34	40	30	33	23	34	42	29
	AVERAGE	36	41	30	33	23	34	41	30
230	D1	12	27	25	34	22	33	34	30
	D2	13	28	25	20	16	27	35	28
	AVERAGE	13	28	25	27	19	30	35	29
234	D1	30	40	30	29	20	32	37	26
	D2	36	42	29	31	21	30	38	26
	AVERAGE	33	41	30	30	21	31	38	26
237	D1	18	31	26	17	21	30	36	25
	D2	19	30	25	17	23	29	37	26
	AVERAGE	19	31	26	17	22	30	37	26
242	D1	27	32	23	23	16	24	35	22
	D2	28	35	25	25	15	25	34	22
	AVERAGE	28	34	24	24	16	25	35	22
244	D1	28	35	26	23	16	25	34	21
	D2	29	34	24	24	15	24	33	22
	AVERAGE	29	35	25	24	16	25	34	22
266	D1	27	34	25	27	20	29	33	25
	D2	29	31	26	26	18	28	30	23
	AVERAGE	28	33	26	27	19	29	32	24
391	D1	35	40	26	12	17	29	40	6
	D2	34	40	22	12	15	27	46	6
	AVERAGE	35	40	24	12	16	28	43	6
407	D1	32	26	35	35	9	12	26	23
	D2	35	25	34	36	10	13	33	24

	AVERAGE	34	26	35	36	10	13	30	24
468	D1	32	37	34	36	30	34	40	16
	D2	31	34	33	33	32	30	41	18
	AVERAGE	32	36	34	35	31	32	41	17

Antimicrobial susceptability testing for S. aureus isolates from human blood

Sample No.	Diameter (mm)	FOX 30µg	AMP 10µg	CIP 5µg	ERY 15µg	GENT 10µg	TET 30µg	AMC 30µg	SXT 25µg
ATCC 25923	D1	28	28	25	25	28	27	25	29
	D2	30	24	26	23	28	23	25	29

	AVERAGE	29	26	26	24	28	25	25	29
3	D1	28	27	26	24	19	8	25	30
	D2	28	25	26	24	19	9	25	30
	AVERAGE	28	26	26	27	19	9	25	30
4	D1	28	16	23	28	20	8	22	24
	D2	27	16	23	26	20	8	22	24
	AVERAGE	28	16	23	27	20	8	22	24
8	D1	28	18	28	25	19	9	24	28
	D2	28	18	26	25	19	9	24	26
	AVERAGE	28	18	27	25	19	9	24	27
10	D1	28	20	30	27	20	8	30	24
	D2	28	20	30	25	20	9	30	24
	AVERAGE	28	20	30	26	20	9	30	24
11	D1	30	18	25	27	21	11	25	22
	D2	30	18	25	25	19	11	25	22
	AVERAGE	30	18	25	26	20	11	25	22
12	D1	30	20	24	30	23	12	24	20
	D2	28	18	24	30	23	10	24	21
	AVERAGE	29	19	24	30	23	11	24	21
13	D1	30	18	24	29	20	9	28	6
	D2	29	19	25	28	20	9	29	6
	AVERAGE	30	19	25	29	20	9	29	6
14	D1	30	22	28	30	22	29	30	6
	D2	30	23	29	30	21	28	30	6
	AVERAGE	30	23	29	30	22	29	30	6
15	D1	32	18	28	6	23	30	26	6
	D2	31	16	27	6	22	28	25	6
	AVERAGE	32	17	28	6	23	29	26	6
16	D1	30	21	32	28	22	29	30	6
	D2	29	20	32	28	21	28	30	6
	AVERAGE	30	21	32	28	22	29	30	6
18	D1	30	20	28	6	22	10	30	6
	D2	29	20	26	6	21	10	30	6
	AVERAGE	30	20	27	6	22	10	30	6
19	D1	29	20	29	30	21	6	27	18

	D2	30	21	28	29	21	6	26	17
	AVERAGE	30	21	29	30	21	6	27	18
22	D1	31	21	30	29	22	30	30	6
	D2	30	21	31	28	21	31	30	6
	AVERAGE	31	21	31	29	22	31	30	6
24	D1	30	19	30	29	20	30	31	8
	D2	31	18	31	30	21	31	30	9
	AVERAGE	31	19	31	30	21	31	31	9
25	D1	35	19	28	30	25	30	25	26
	D2	33	17	27	31	25	29	25	25
	AVERAGE	34	18	28	31	25	30	25	26
27	D1	29	20	30	27	22	9	29	23
	D2	28	21	31	26	21	8	30	24
	AVERAGE	29	21	31	27	22	9	30	24
28	D1	32	22	24	30	24	33	33	6
	D2	31	22	25	30	23	32	31	6
	AVERAGE	32	22	25	30	24	33	32	6
29	D1	30	24	27	30	24	10	25	25
	D2	30	22	26	30	23	10	26	24
	AVERAGE	30	23	27	30	24	10	26	25
30	D1	30	20	29	30	21	9	29	6
	D2	29	20	28	30	20	9	28	6
	AVERAGE	30	20	29	30	21	9	29	6
31	D1	29	18	26	25	22	9	24	6
	D2	28	19	25	26	22	9	24	6
	AVERAGE	29	19	26	26	22	9	24	6
32	D1	30	19	28	30	23	30	27	6
	D2	29	17	28	32	25	32	26	6
	AVERAGE	30	18	28	31	24	31	27	6
33	D1	27	18	26	28	18	28	22	24
	D2	25	18	26	27	19	26	23	24

	AVERAGE	26	18	26	28	19	27	23	24
34	D1	28	14	27	6	20	30	23	6
	D2	28	12	26	6	21	29	23	6
	AVERAGE	28	13	27	6	21	30	23	6
35	D1	25	17	28	6	19	28	26	6
	D2	25	17	27	6	18	27	25	6
	AVERAGE	25	17	28	6	19	28	26	6
36	D1	29	15	25	30	20	30	23	22
	D2	28	14	26	29	21	29	23	21
	AVERAGE	29	15	26	30	21	30	23	22
37	D1	30	15	28	30	18	10	22	23
	D2	29	16	28	29	18	9	22	24
	AVERAGE	30	16	28	30	18	10	22	24
38	D1	29	16	23	28	19	29	22	24
	D2	28	16	22	27	19	28	22	24
	AVERAGE	29	16	23	28	19	29	22	24
39	D1	25	13	27	24	18	8	23	6
	D2	25	14	26	25	17	8	24	6
	AVERAGE	25	14	27	25	18	8	24	6
78	D1	26	20	28	28	18	29	26	6
	D2	26	21	26	29	18	29	26	6
	AVERAGE	26	21	27	29	18	29	26	6
79	D1	28	13	28	26	18	28	22	11
	D2	29	12	27	27	19	29	21	10
	AVERAGE	29	13	28	27	19	29	22	11
80	D1	21	13	30	28	19	30	23	24
	D2	21	12	29	29	19	29	22	22
	AVERAGE	21	13	30	29	19	30	23	23
92	D1	26	13	26	27	19	26	22	10
	D2	16	14	26	26	19	25	23	9
	AVERAGE	21	14	26	27	19	26	23	10
93	D1	21	20	30	30	22	30	26	25
	D2	21	20	29	30	23	29	25	24
	AVERAGE	21	20	30	30	23	30	26	25
103	D1	23	18	29	27	20	28	24	20

D2	19	18	29	27	19	29	24	19

	AVERAGE	21	18	29	27	20	29	24	20
104	D1	26	16	27	28	16	29	22	22
	D2	26	14	26	28	12	27	22	22
	AVERAGE	26	15	27	28	14	28	22	22
115	D1	14	6	6	6	12	26	12	6
	D2	15	6	6	6	12	27	12	6
	AVERAGE	15	6	6	6	12	27	12	6
469	D1	14	6	6	6	12	6	11	6
	D2	15	6	6	6	11	6	12	6
	AVERAGE	15	6	6	6	12	6	12	6
470	D1	11	6	12	17	10	24	10	6
	D2	10	6	12	18	9	23	9	6
	AVERAGE	11	6	12	18	10	24	10	6
471	D1	16	6	6	20	11	25	12	6
	D2	15	6	6	19	10	24	13	6
	AVERAGE	16	6	6	20	11	25	13	6
475	D1	10	6	6	6	7	7	12	6
	D2	9	6	6	6	6	8	11	6
	AVERAGE	10	6	6	6	7	8	12	6
476	D1	11	7	6	13	10	23	14	6
	D2	12	8	6	14	9	22	13	6
	AVERAGE	12	8	6	14	10	23	14	6
479	D1	10	6	10	11	9	21	15	15
	D2	12	7	11	10	8	20	14	16
	AVERAGE	11	7	11	11	9	21	15	16
482	D1	12	6	6	14	10	22	10	6
	D2	11	6	6	13	9	23	11	6
	AVERAGE	12	6	6	14	10	23	11	6
484	D1	11	6	6	16	10	25	12	6
	D2	12	6	6	15	11	25	13	6
	AVERAGE	12	6	6	16	11	25	13	6
485	D1	9	6	8	15	9	23	12	14
	D2	10	6	9	16	10	23	11	16
	AVERAGE	10	6	9	16	10	23	12	15
486	D1	25	14	25	25	21	26	30	25

	D2	26	14	25	25	21	25	31	26
	AVERAGE	26	14	25	25	21	26	31	26
487	D1	7	6	10	14	10	20	10	6
	D2	9	6	9	14	9	21	10	6
	AVERAGE	8	6	10	14	10	21	10	6
488	D1	10	6	6	6	10	7	12	6
	D2	11	6	6	6	11	8	11	6
	AVERAGE	11	6	6	6	11	8	12	6
490	D1	12	6	7	16	9	23	11	6
	D2	11	6	6	16	10	22	12	6
	AVERAGE	12	6	7	16	10	23	12	6
491	D1	14	10	26	26	19	25	20	28
	D2	15	11	22	25	20	24	21	27
	AVERAGE	15	11	24	26	20	25	21	28
493	D1	13	7	7	6	12	6	11	6
	D2	12	6	6	6	12	6	12	6
	AVERAGE	13	7	7	6	12	6	12	6
494	D1	12	6	6	16	10	24	12	6
	D2	11	6	6	15	11	24	11	6
	AVERAGE	12	6	6	16	11	24	12	6
495	D1	26	18	28	29	24	30	25	33
	D2	27	19	27	30	24	31	26	32
	AVERAGE	27	19	28	30	24	31	26	33

KEY: Resistant

Intermediate Resistant

Susceptible