Phenotypic and Molecular Characterizations of *Staphylococcus aureus* Isolates from Human and Dairy Cows in Mekele, Northern Ethiopia

Alem Abrha (BSc, MSc)

A PhD thesis submitted to the Department of Microbiology, Immunology and Parasitology (DMIP), College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in Medical Microbiology

March, 2019

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Phenotypic and Molecular Characterizations of *Staphylococcus aureus* Isolates from Human and Dairy cows in Mekele, Northern Ethiopia

By

Alem Abrha (BSc, MSc); DMIP, College of Health Sciences, Addis Ababa University, Ethiopia

Supervisors

Daniel Asrat (MD, MSc, PhD)

Professor, DMIP, College of Health Sciences, Addis Ababa University, Ethiopia

Yimtubezinash Woldeamanuel (MD, MSc, PhD)

Associate Professor, DMIP, College of Health Sciences, Addis Ababa University, Ethiopia

Shu-Hua Wang (MD, PharmD, MPH & TM)

Associate Professor, Infectious Disease Division, Internal Medicine Department, College of Medicine, the Ohio State University, USA

Wondwossen A. Gebreyes (DVM, PhD, DACVPM)

Professor, Molecular Epidemiology; Director, Global Health Programs; College of Veterinary Medicine; the Ohio State University; USA

Collaborator

Kibrom Gebre Selassie (MD)

Assistant Professor of surgery, Mekele University, Mekele, Northern Ethiopia

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to all my advisors Professor Daniel Asrat, Dr. Yimtubezinash Woldeamanuel, Prof. Shu-Hua Wang and Professor Wondwossen Gebreyes for their unreserved support throughout my PhD study. Their guidance helped me in all the time of research, writing of this thesis and my career. I could not have imagined having better advisors and mentors for my PhD study.

In addition to his tremendous guidance, I would also like to extend my thanks to professor Wondwossen for providing me an opportunity to join his laboratory (Infectious Diseases Molecular Epidemiology Laboratory, IDMEL) at the college of veterinary medicine, Ohio State University, USA. He gave me full laboratory access and covered all the expenses. Similarly, my sincere thanks also goes to Dr. Barry Kreiswirth and Mr. José Mediavilla at the Public Health Research Institute of The State University of New Jersey, USA for generously conducted *spa* typing of all the *S. aureus* isolates. Without their precious support it would not be possible to conduct this research.

My heartfull thanks also goes to Addis Ababa University, Aksum University and Ohio State University for funding this PhD project. I would also thank CDT Africa for covering the cost for the external examiner.

Besides my advisors, my special thanks also go to Dr. Tunika Okatcha, who was the laboratory manager of IDMEL, for her full support during my stay in and outside the laboratory.

I would also like to thank members of the Department of Microbiology, Immunology and Parasitology; College of Health Sciences, Addis Ababa University for their insightful comments and encouragement.

My sincere thanks also go to members of the Microbiology laboratory of Ayder Referal Hospital, Mekele, Ethiopia for being by my side tirelessly throughout the sample collection and phenotypic characterization of this study.

Last but not the least; I would like to express my appreciation to my beloved wife Mrs. Zewditu Mulaw who was always my support and for her patience.

TABLE OF CONTENTS

CONTENTS **PAGE**

ACKNOWLEDGEMENTS i
TABLE OF CONTENTS ii
LIST OF TABLES iv
LIST OF FIGURES iv
ABBREVIATIONS vi
ABSTRACT viii
CHAPTER ONE: INTRODUCTION 1
1.1. General Introduction 1
1.2. Literature Review 3
1.2.1. The Genus *Staphylococcus* 3
1.2.2. The Genome of *S. aureus* 4
1.2.3. *Staphylococcus aureus* colonization 7
1.2.4. Virulence factors and Pathogenesis of *S. aureus* infection 8
1.2.5. Clinical features of *S. aureus* infection 14
1.2.6. Epidemiology of Staphylococcal SSTIs in human and mastitis in cattle 17
1.2.7. Laboratory Methods for diagnosis of *S. aureus* Infection 21
1.2.8. Treatment of *S. aureus* infections 25
1.2.9. Drug resistance in *S. aureus* 26
1.2.10. Prevention and control of *S. aureus* infections 29
1.3. Statement of the problem 29
1.4. Significance of the study 31
1.5. Hypothesis 32
1.6. OBJECTIVES OF THE STUDY 33
CHAPTER TWO: METHODS AND MATERIALS 34
2.1. Study area 34
2.2. Study design and period 35
2.3. Study Population 35
2.4. Sample size and sampling technique 35
2.5. Operational Definitions 37

2.6. Data collection 37
2.7. Specimen Collection, Handling and Transport 38
2.8. Laboratory Procedures 39
2.8.1. Culture and Identification of *S. aureus* 41
2.8.2. Antimicrobial Susceptibility Testing 41
2.8.3. Molecular characterizations of *S. aureus* Isolates 42
2.9. Quality Control 50
2.10. Data analysis 50
2.11. Ethical consideration 51
CHAPTER THREE: RESULTS 52
3.1. Sociodemographic characteristics of the study participants 52
3.2. Isolation rate of Staphylococcus aureus 55
3.3. Antimicrobial susceptibility data 56
3.4. Toxin genes profile of *S. aureus* isolates 59
3.5. *mec* genes carriage and *SCCmec* typing 65
3.6. *spa* typing result 66
3.7. Probable clonal complex (CC) 68
3.8. Major toxin genes possession by the top eight *spa* types 70
CHAPTER FOUR: DISCUSSION 71
LIMITATIONS OF THE STUDY 85
CONCLUSIONS 85
RECOMMENDATIONS 86
REFERENCES 87
ANNEXES 104
Annex I: Information sheets 104
Annex II: Concent forms 128
Annex III: Questionnaire/ Data collection forms 140
Annex IV: Culture and antimicrobial susceptibility data 144
Annex V: Molecular Characterizations Procedures and Data 145
Annex VI: Laboratory procedures and Interpretations 152
Annex VII: Material Transfer Agreement (MTA) 162

LIST OF TABLES

TABLES **PAGE**

Table 1.1: The coagulase-positive *Staphylococci* 4
Table 2.1. Control strains and primer pairs for the multiplex PCR to detect *spa, pvl, mecA, mecC* genes of *S. aureus* isolated from humans and animals, Mekele, Ethiopia.........44
Table 2.2: Control strains and primer pairs for the multiplex PCR to detect superantigen genes of *S. aureus* isolated from humans and animals, Mekele, Ethiopa 45
Table 2.3: Control strains and primers for *S. aureus* SCC*mec* typing of MRSA isolated from humans and animals, Mekele, Northern Ethiopia 47
Table 2.4: Interpretation of MPCR1 and MPCR2 for the assignment of SCC*mec* types 48
Table 3.1. Sociodemographic characteristics of study participants with SSTIs in Ayder Referral Hospital, Mekele, Northern Ethiopia 53
Table 3.2: Characteristics of Dairy cows from 67 small and large scale dairy farms in Mekele, Northern Ethiopia..54
Table 3.3: Sociodemographic characteristics of Dairy farm workers from 67 small and large scale dairy farms in Mekele, Northern Ethiopia 55
Table 3.4: Frequency of *S. aureus* isolation from the different study groups in Mekele, Northern Ethiopia...56
Table 3.5: Antimicrobial susceptibility profile of 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia 57
Table 3.6: Antibiogram profile of 193 *S. aureus* isolates from humans and dairy cows in Mekele, Northern Ethiopia..58
Table 3.7. Percentage of toxin genes positivity of the 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia 59
Table 3.8: Toxin genes profile of the 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia..60
Table 3.9: Combinations of toxin genes carried by *S. aureus* isolates from different sources, Mekele Northern Ethiopia..61
Table 3.10. Combination of staphylococcal enterotoxin (*se*) genes of the 193 *S. aureus* isolates from Mekele, Northern Ethiopia 64
Table 3.11: Combination of classical enterotoxin genes carried by the 193 *S. aureus* isolates from Mekele, Northern Ethiopia 65
Table 3.12. Pattern of *spa* types of the 190 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia..67
Table 3.13: Probable Clonal Complex of 190 *S. aureus* isolates from different sources, Mekele Northern Ethiopia..69
Table 3.14. Comparison of *spa* types with the major toxin genes of *S. aureus* isolates from Mekele, Northern Ethiopia..70

LIST OF FIGURES

FIGURES **PAGE**

Figure 1.1: *S. aureus* virulence factors 9

Figure 1.2: Gram stain from tissue with *S. aureus* infection 21

Figure 2.1: Map of Ethiopia: Tigray region administrative map..34

Figure 2.2: Laboratory flow chart for isolation, phenotypic and molecular characterization of *S. aureus* 40

Figure 3.1: Resistance pattern out of the nine antimicrobials tested for the 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia. 58

Figure 3.2: Toxin gene carriage out of the 11 target genes by the 193 *S. aureus* isolates from Mekele, Northern Ethiopia 62

Figure 3.3: Percentage of probable CCs based on *spa* sequence of 190 *S. aureus* isolates Mekele, Northern Ethiopia 69

ABBREVIATIONS

ADAM10:	A Disintegrin and Metalloproteinase 10
APCs:	Antigen Presenting Cells
ATCC:	American Type Culture Collection
CA-MRSA:	Community Acquired Methicillin-Resistant *S. aureus*
CC:	Clonal Complex
CCR:	Cassette Chromosome Recombinase
CDC:	Centers for Disease Control and Prevention
CHIPS:	Chemotaxis Inhibitory Protein of *Staphylococcus aureus*
Clf:	Clumping Factor
CLSI:	Clinical and Laboratory Standards Institute
CMT:	California Mastitis Test
CoNS:	Coagulase-Negative *Staphylococci*
CoPS:	Coagulase-Positive *Staphylococci*
CV:	Core Variable Genes
DNA:	Deoxyribonucleic Acid
DNase:	Deoxyribonuclease
D-test:	Double-Disk Diffusion Test
E-test:	Epsilometer Test
ET:	Exfoliative Toxin
FDA:	Food and Drug Administration
HA-MRSA:	Healthcare Associated Methicillin-Resistant *S. aureus*
LA-MRSA:	Livestock Acquired Methicillin-Resistant *S. aureus*
MGEs:	Mobile Genetic Elements
MHC:	Major Histocompatibility Complex
MIC:	Minimum Inhibitory Concentration
MLST:	Multi-Locus Sequence Typing
MRSA:	Methicillin-Resistant *S. aureus*
MSCRAMMs:	Microbial Surface Components Recognizing Adhesive Matrix Molecules

ABBREVIATIONS

MSSA:	Methicillin-Susceptible *S. aureus*
NMC:	National Mastitis Council
PBP:	Penicillin Binding Protein
PCR:	Polymerase Chain Reaction
PFGE:	Pulsed-Field Gel Electrophoresis
PSM:	Phenol-Soluble Modulin
PVL:	Panton-Valentine Leukocidin
SAgs:	Superantigens
SaPIs:	*S. aureus* Pathogenicity Islands
SCC*mec*:	Staphylococcal Cassette Chromosomes *Mec*
Se*l*:	Staphylococcal Enterotoxin-*like*
SEs:	Staphylococcal Enterotoxins
spa:	Staphylococcal Protein A
SSIs:	Surgical Site Infections
SSSS:	Staphylococcal Scalded Skin Syndrome
SSTIs:	Skin and Soft Tissue Infections
ST:	Sequence Type
TSS:	Toxic Shock Syndrome
TSST:	Toxic Shock Syndrome Toxin
VISA:	Vancomycin Intermediate *S. aureus*
VRSA:	Vancomycin Resistant *S. aureus*

ABSTRACT

Introduction: *Staphylococcus aureus* is one of the common colonizers of human and several animal species with the capacity to cause serious diseases. It is one of the global public health priority pathogens due to its ubiquitous nature, ability to cause life-threatening diseases and continuous evolvement of drug resistance. The emergence and spread of multidrug resistant strains, mainly MRSA, initially in the health care setting, later in the community and recently in the veterinary side with a capacity of transmission to human is of special concern. Several molecular techniques have been used successfully for reliable identification, determination of specific virulence and drug resistance genes and to understand spread and distribution of specific *S. aureus* strains. In Ethiopia, despite several reports on phenotypic studies of *S. aureus* isolates from human and animals, data on molecular characterizations is scarce.

Objectives: To isolate *S. aureus* from human skin and soft tissue infections, milk of dairy cows and nares of dairy farm workers in Mekele, Northern Ethiopia; and then to determine the antimicrobial resistance pattern, virulence genes profile and genotypic diversity and relatedness of the isolates.

Methods: A cross sectional study was conducted during period of March 2016 to August 2017 G.C. A total of 811 non-duplicate specimens (355 wound/pus swabs from human patients with skin and soft tissue infections [SSTIs] attending Ayder referral hospital, 71 nasal swabs from dairy farmers and 384 milk samples from dairy cows) were collected to isolates *S. aureus*. Culture and identification of *S. aureus* was performed using standard laboratory procedures. Antimicrobial susceptibility testing was performed following the CLSI guideline. All phenotypic characterizations of *S. aureus* were done at the Microbiology laboratory of Ayder Referral Hospital, Mekele, Ethiopia. All *S. aureus* isolates were stored at -70^0C and shipped to USA for Molecular characterizations at the Infectious Diseases Molecular Epidemiology Laboratory (IDEML) of the Ohio State University and the Public Health Research Institute of the State University of New Jersey, USA. Molecular characterizations include confirmation of *S. aureus* by *nuc* detection,

*mecA/mec*C detection, toxin genes profiling (*pvl,* nine *se* genes and *tsst-1*), *spa* typing and SCC*mec* typing.

Results: From the 811 samples, 193 (23.8%) were positive for *S. aureus* using phenotypic and molecular methods; where 123 (63.7%) were from human SSTIs, 22 (11.4%) from nares of dairy farmers and 48 (24.9%) from milk of Dairy cows. Overall, high percentage of resistance was observed for Penicillin (92.7%); however, no resistance was found for Vancomycin, Daptomycin and Rifampin. Four (2.1%) of the 193 isolates were MRSA where all were from human and were both cefoxitin resistant and *mecA* positive. Two of them carried SCC*mec* type III and the other two SCC*mec* type IV. Moreover, 26% of *S. aureus* isolates were multidrug resistant. Regarding toxin genes carriage, 66.8% of the total isolates possessed at least one of the 11 targeted toxin genes. Of the 11 genes, *pvl* was the most frequently detected (carried by 36.8%) followed by *seg* and *sei* (29% each), *seb* (19.2%), *sea* (16%) and *sec* (15%). Interestingly, *pvl* was found significantly higher *($p<0.001$)* on isolates from SSTI (53.7%) compared to nasal (13.6%) and cows' milk (4.2%) isolates. In addition, *tsst-1* was found in human isolates only. The *spa* typing yielded 61 different types among the 190 successfully typed isolates. Out of these, five were novel *spa* types, namely *spa* type t17828, t17829, t17830, t17831 and t17832. The most dominant *spa* type among the SSTI isolates was t355 (19%) followed by t306 (8.3%); whereas in milk *spa* type t042 (61.7%) was the dominant one followed by t2856 (19.1%). The present study also noted that *spa* type t042, t306, t085, t2856 and t5338 were detected from both human and dairy cows. Based on the *spa* sequence information, 90.5% of the total *S. aureus* were assigned in to 12 probable clonal complexes (CCs), namely CC1, CC5, CC8, CC15, CC22, CC25, CC80, CC81, CC88, CC121, CC152 and CC239. The leading CC among the human clinical isolates was CC152 (20.7%) followed by CC15 (19%) and CC5 (16.5%). However, most of the *S. aureus* isolates from dairy cows (83%) were CC80 followed by CC5 (6.4%).

Conclusion: In the present study, *S. aureus* was isolated from skin and soft tissue infections, nares of farm workers and milk of dairy cows. Highest resistance was observed against penicillin. No resistance was observed against Vancomycin, Daptomycin and Rifampin. Multidrug resistance was observed in a quarter of the isolates. *mecA* positive *S. aureus*

(MRSA) strains were of human origin. Majority of *S. auresus* isolates possessed at least 1 of the 11 toxin genes. The *spa* typing of *S. aureus* resulted in 61 different types and showed diversity. Based on the *spa* sequence information, *S. aureus* isolates were assigned into 12 probable clonal complexes (CCs). Among these CCs, CC1, CC5, CC15 and CC80 were shared between human and bovine isolates. Findings of the present study indicate that *S. aureus* is a significant cause of skin and soft tissue infections in human and intramammary infections in dairy cows. Hence, measures to reduce/prevent infection such as improving infection prevention practices in the hospital, keeping hygiene of the dairy cows and educating the community to practice the best possible personal hygiene are recommended. Further study should be conducted in different regions of the country to have conclusive data for policy makers, clinicians and researchers.

Key words: *Staphylococcus aureus*, MRSA, Antimicrobial resistance, toxin genes, *spa* typing, clonal complex, Ethiopia

CHAPTER ONE: INTRODUCTION

1.1. General Introduction

Staphylococcus aureus (*S. aureus*) is a spherical gram positive coccus of about 1 μm in diameter and usually occurs in irregular grapelike clusters in Gram stained smears. It is non-motile, non-spore forming, facultative anaerobic, capable of producing catalase and coagulase [Hennekinne *et al.*, 2012]. It can grow at a temperature range of 15 to 45 ^{0}C and as high as 15% NaCl concentrations. It is resistant to high osmolarity, detergents, and alcohol [Missiakas and Schneewind, 2013]. It possesses a variety of virulence factors that mediate adherence, lyse host cells, tissue destruction and spread, evade and manipulate immune responses. The most common toxins produced by *S. aureus* include hemolysins, leukotoxins (e.g. Panton-Valentine Leukocidin), exfoliative toxins, enterotoxins, and toxic shock syndrome toxin (TSST) [Grumann *et al.*, 2014; Otto, 2012] .

S. aureus is capable of colonizing and infecting human and several animal species including livestock, wildlife, and companion animals [Paterson *et al.*, 2014]. In addition, it survives well in dry conditions and can persist for long periods on environmental surfaces [Lin *et al.*, 2016]. *S. aureus* colonizes approximately a third of world population, with the most common carriage site being in the anterior nares [Otto *et al.*, 2014]. On the other hand, *S. aureus* is also a virulent pathogen capable of infecting almost every tissue of the body and causing infections ranging from minor skin infections to life-threatening infections; such as bacteremia, endocarditis, necrotizing pneumonia and toxic shock syndrome (TSS) [Lin and Peterson, 2010]. Veterinary significances include bovine mastitis in dairy cattle, lameness in poultry, and severe and lethal infections in farmed rabbits. Besides from an animal welfare and economic perspective, infected animals can serve as a reservoir for humans infections [Paterson *et al.*, 2014].

Continuous evolution of antimicrobial resistance makes *S. aureus* as one of the major public health threat pathogens worldwide [Ortega et al., 2010]. The emergence and spread of methicillin-resistant *S. aureus* (MRSA) is of great significance [Ortega *et al.*, 2010].

Initially, MRSA emerged and was restricted in the healthcare setting causing nosocomial infections. Later in the 1990s, it was identified in the community causing infections in relatively young and healthy individuals lacking contact with healthcare [Paterson *et al.*, 2014]. More recently, livestock acquired (LA) MRSA has spread extensively in livestock animals and people having occupational contact with these animals [Verkade and Kluytmans, 2014]. The adaptation and evolution of *S. aureus* is largely due to the acquisition of mobile genetic elements (MGEs) that carry virulence and resistance genes [McCarthy *et al.*, 2012]. *S. aureus* has also developed resistance even to vancomycin, the treatment of choice for MRSA, and to multiple other antibiotics, including macrolides, aminoglycosides and fluoroquinolones [Marasa *et al.*, 2014].

Several molecular techniques have been used successfully to describe the global population structure of *S. aureus*; to study their subsequent worldwide dissemination and evolution; to provide a framework for the description of the major lineages in different countries; and to monitor their emergence, dispersal and decline in different settings [Price *et al.*, 2013]. Not unexpectedly, data regarding the molecular characteristics and epidemiology of *S. aureus* is limited in the developing world, especially sub-Saharan Africa.

Previous studies in Ethiopia documented *S. aureus* as a major pathogen of community acquired infections [Azene and Beyene, 2011; Mulu *et al.*, 2006], hospital acquired infections [Dessalegn *et al.*, 2014; Melaku *et al.*, 2012; Mulu *et al.*, 2012] and dairy farms [Abdella, 1996; Abera *et al.*, 2012; Getahun *et al.*, 2008; Haftu *et al.*, 2012]. However, there is few available data regarding molecular epidemiology, molecular characteristics of drug resistance genes and toxins, and distribution of *S. aureus* genotypes between human and animals in Ethiopia [Seyoum *et al.*, 2016; Tarekgne *et al.*, 2016; Tigabu *et al.*, 2015]. Ethiopia is a country where many of its population live in close contact with domestic animals; the extent of transmission and potential ongoing outbreak is unknown. Treatment is mainly given empirically due to lack of diagnostic capabilities and adequate laboratory facilities. Empiric use of antibiotics can lead to inappropriate use of antibiotics, increase drug resistance and more importantly, delay in treatment and care of human and animal.

Therefore, the present study was conducted to determine the prevalence, drug resistance pattern, toxin characteristics and genotypic diversity of *S. aureus* in the hospital setting, community and dairy farms in Ethiopia inorder to have better understanding, improve public health and explore future research areas.

1.2. Literature Review

1.2.1. The Genus *Staphylococcus*

A. Historical perspectives

Using Koch's staining methods (aniline-violet stain); Sir Alexander Ogston (1844-1929) observed clusters of round organisms in pus taken from human abscesses in 1880. Then, he named the grouped form of the micrococcus as '*Staphylococcus'* (Greek, *staphyle* for bunch of grapes and *kokkos* for berry) in 1882 [Ogston, 1882]. In 1884, the German surgeon Anton J. Rosenbach (1842-1923) isolated two strains of *Staphylococci* and named them for the pigmented appearance of their colonies as *Staphylococcus aureus* (Latin, *aurum* for gold) and *Staphylococcus albus* (Latin, *albus* for white). *S. albus* was later renamed *S. epidermidis* because of its ubiquity on human skin (www.antimicrobe.org).

B. General characteristics

The genus *Staphylococcus* consists of Gram positive, spherical cocci of about 1 μm in diameter [Plata *et al.*, 2009]. When observed under the microscope; they occur in pairs, short chains or grape-like clusters [Hennekinne *et al.*, 2012]. The characteristic grape-like clustering of *Staphylococci* results from the incomplete separation of daughter cells following division in three alternating perpendicular planes [Missiakas and Schneewind, 2013]. The *Staphylococci* are also non-motile, non-spore forming, facultative anaerobic, catalase positive organisms. There are more than 50 species and subspecies of *Staphylococci* which are ubiquitous in the air, dust, sewage, water, environmental surfaces, humans and animals [Hennekinne *et al.*, 2012]. Based on their ability to produce coagulase, *Staphylococci* are classified into coagulase positive and coagulase negative [Hennekinne *et al.*, 2012] (Table 1.1).

Table 1.1: The coagulase-positive *Staphylococci* (adapted from [Hennekinne *et al.*, 2012])

Species	Main source
S. aureus ssp. aureus	Humans, animals
S. aureus ssp. anaerobius	Sheep
S. intermedius	Dog, horse, mink, pigeon
S. pseudintermedius	Dog, cat
S. delphini	Dolphin
S. schleiferi ssp. coagulans	Dog (external ear)
S. lutrae	Otter

Among the coagulase-positive *Staphylococci* (CoPS), *S. aureus* is the most virulent species. *S. aureus* usually forms medium sized "golden" colonies on enriched medium (due to the carotenoids) and β hemolytic colonies on blood agar plates [Plata *et al.*, 2009]. It can grow at a temperature range of 15 to 45 ^{0}C and as high as 15% NaCl concentrations. It is resistant to high osmolarity, detergents, as well as inadequate alcohol disinfection [Missiakas and Schneewind, 2013].

1.2.2. The Genome of *S. aureus*

The first *S. aureus* whole genome sequence project, which was completed in 2001, revealed that the chromosome of *S. aureus* is about 2.8 Mb with low G+C content (~33%) [Kuroda *et al.*, 2001]. Since then several whole genome sequence projects have been completed and deposited in the GenBank (http://www.ncbi.nlm.nih.gov/genbank). Comparison of this huge amount of data revealed that the genome structure of *S. aureus* has three principal components: a backbone of core genes, core variable (CV) genes and Mobile genetic elements (MGEs) [Stefani *et al.*, 2012].

The backbone of genes accounts for about 75% of the *S. aureus* genome. They are highly conserved shared by more than 95% of strains [Grumann *et al.*, 2014]. The products of these genes are involved in fundamental functions of the cell such as metabolism, growth and replication [Price *et al.*, 2013]. The core variable (CV) genes, accounted for about 10% of

the genome, are found dispersed throughout the backbone. The CV genes are variably distributed and their distribution pattern defines *S. aureus* lineages [Stefani *et al.*, 2012]. The CV genes include most surface-associated genes and regulator genes. They are encoded on the bacterial chromosome and are, therefore, typically stable and transferred vertically [Grumann *et al.*, 2014].

The Mobile genetic elements (MGEs), which account for 15% of *S. aureus* genome are gained and lost at high frequencies through horizontal gene transfer [Price *et al.*, 2013]. The MGEs carry most of the *S. aureus* virulence factors and antibiotic resistance genes [Shearer *et al.*, 2011]. The MGEs in *S. aureus* include prophage, *S. aureus* pathogenicity islands (SaPIs), plasmids, transposons and staphylococcal cassette chromosomes (SCC) [Lindsay, 2014; McCarthy *et al.*, 2012]

A. Plasmids

Most *S. aureus* strains harbor at least one plasmid carrying resistance to antibiotics, metals, antiseptics, and disinfectants, as well as virulence genes. These plasmids may be horizontally transferred through conjugation and/or transduction [Shearer *et al.*, 2011]. Conjugative plasmids can spread readily among *Staphylococcus* strains and to and from other genera such as *Enterococcus* species [Shearer *et al.*, 2011]. The plasmid encoded factors in *S. aureus* include the β-lactamase encoded by *bla*Z gene, *van*A-mediated resistance to Vancomycin encoded by *Tn*1546, resistance to a variety of highly toxic organic and inorganic ions (such as cadmium, mercury, arsenate, etc.), exfoliative toxin B encoded by pRW001 [Malachowa and DeLeo, 2010].

B. Phages

Phages are widespread in *S. aureus,* providing a source of genetic diversity, where each strain contains at least one prophage and many strains carry up to four. The *S. aureus* phages are DNA phages whereby the phage DNA integrates into the staphylococcal genome as a prophage [Deghorain and Van Melderen, 2012]. Their genome size extends from about 15-130 kb [Kwan *et al.*, 2005]. The Phages encoded virulence factors in *S. aureus* include staphylokinase (*sak*), the chemotaxis inhibitory protein (*chp*), the staphylococcal inhibitor of

complement (*scn*), several superantigens (*sea*, *seg*, *sek*, *sek2*, *sep*, *seq*), the Panton-Valentine leukocidin (*lukF-PV*, *lukS-PV*) and related leukocidins (*lukM*, *lukF*), exfoliative toxin A (*eta*) [Deghorain and Van Melderen, 2012] and the immune evasion cluster (*Chp, Sak, and Scn*) associated with human specificity [McCarthy *et al.*, 2012].

C. Staphylococcal pathogenicity islands (SaPIs)

Staphylococcal pathogenicity islands (SaPIs) are 14–17 kb [Malachowa and DeLeo, 2010] residing normally at specific sites in the chromosome of their host and remain quiescent under the control of a master repressor. However, in the presence of specific "helper" bacteriophages (e.g. bacteriophage 80α), they become de-repressed and enabled to express their integrase and excision genes [Juhas, 2013; Ram *et al.*, 2012]. During mobilization, the SaPI genomes are packaged into transducing particles encoded by the helper phage [Dearborn and Dokland, 2012]. Among the SaPIs encoded *S. aureus* factors are enterotoxins and toxic shock syndrome toxin (TSST) [Malachowa and DeLeo, 2010].

D. Transposons

S. aureus posesses more than ten transposons, the majority of which carry antibiotic resistance genes. Transposons usually carry a transposase gene and other genes often are antibiotic resistance determinants. The transposase participates during recombination required for transposition [Plata *et al.*, 2009].

E. Staphylococcal cassette chromosome *mec* (SCC*mec)*

The SCC*mec* are 20 to 60 kb found integrated into the staphylococcal chromosome at a specific site called the integration site sequence (ISS) within the 3' end of the *orf*X gene. Each SCC*mec* element carries a cassette chromosome recombinase (*ccr*) and *mec* gene complex. The *mec* genes encode resistance to methicillin and almost all β-lactam antibiotics. However, the *ccr* genes encode serine recombinases that mediate site- and orientation-specific integration and excision of SCC*mec.* There are variable length genes outside of the *ccr* and *mec* gene complexes in the SCC*mec* which are called "Joining" or "J" regions, namely J1, J2 and J3 [Liu *et al.*, 2016a; Shore and Coleman, 2013]. Besides methicillin and penicillin resistances, SCC*mec* often harbors a number of additional genes conferring

resistance to a wide variety of antibiotics, including tetracycline, kanamycin, lincosamide and tobramycin [Juhas, 2013].

To date, 3 *mec* gene types (*mecA, mecB and mecC*) have been identified. The *mecA* gene originally identified in MRSA strain N315 encodes the PBP responsible for β-lactam resistance, PBP2a. The *mecA* gene has been also identified in various staphylococcal species other than *S. aureus*, such as *S epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. fleurettii*. The *mecB*, which was identified in *Macrococcus caseolyticus,* is divergent *mecA* gene homologues with 62% nucleotide sequence identity to *mecA* of N315 [Becker *et al.*, 2014; Ito *et al.*, 2012]. The *mecC* was identified recently in *S. aureus* strain LGA251 and had 69% identity to *mecA* of N315. The *mecC* also encodes PBP2a, which is only ~63% identical at the amino acid level to the *mecA* encoded PBP2a. The emergence of *mecC* poses a diagnostic problem in MRSA, as *mecC* positive strains are usually negative using *mecA* PCR and PBP2a slide agglutination [Paterson *et al.*, 2014]. The naming of *mec* genes as *mecA*, *mecB* and *mecC* is based on the chronological order of discovery [Ito *et al.*, 2012].

1.2.3. *Staphylococcus aureus* colonization

S. aureus usually colonizes the skin and mucosa of human and warm-blooded animals. In addition it can survive well in the environment [Peton and Le Loir, 2014]. It is estimated that about a third of world population is colonized by *S. aureus*, with the most common carriage site being in the anterior nares [Verkade and Kluytmans, 2014]. *S. aureus* can also colonizes other body sites such as mucosal surfaces; including the throat, vaginal wall, and gastrointestinal tract [Liu, 2009]; warm and moist skin folds; such as behind ears, under pendulous breasts, and in the groin [Ibler and Kromann, 2014], the axilla, and the perineum [Price *et al.*, 2013]. Cows may be healthy carriers of *S. aureus* on the teat skin, in the rectum or nasal cavity. However, the principal reservoirs of *S. aureus* in dairy herds are infected udders and teat skin. Hence, infected cows can shed *S. aureus* in milk [Peton and Le Loir, 2014].

Colonization by *S. aureus* can be transient or persistent [Xu and McCormick, 2012]. Transient colonization can be generally described as a single positive culture on a nasal

swab; whereas, persistent colonization is at least two consecutive positive cultures one week apart [Liu, 2009]. The prevalence of nasal colonization by *S. aureus* is particularly high within the general population where about 20% are persistently and 30% transiently colonized [Price *et al.*, 2013]. Difference in carrier state could be related to different ligands for the adhesins of *S. aureus* [Zecconi and Scali, 2013].

Colonization provides a reservoir from which *S. aureus* can be introduced when host defenses are breached; whether by shaving, aspiration, insertion of an indwelling catheter, or surgery [Gordon and Lowy, 2008]. Supporting this, a study demonstrated that about 82% of *S. aureus* isolated from blood-stream infections were identical with those carried in the nose indicating an endogenous source [Eiff *et al.*, 2001]. This can be particularly dangerous in hospital setting if a nasal carrier is immunocompromised and the colonizing strain is drug resistant [Xu and McCormick, 2012]. Moreover, a recent case control study revealed *S. aureus* nasal carriage in 89% and 100% of recurrent and non-recurrent furunculosis, respectively [El-Gilany and Fathy, 2009]. Carriers of *S. aureus* are also two to nine times more likely than non-carriers to have surgical-site infections [Aslam *et al.*, 2013]. Colonization also allows *S. aureus* to be transmitted among individuals in both health care and community settings [Gordon and Lowy, 2008].

1.2.4. Virulence factors and Pathogenesis of *S. aureus* infection

The reason for *S. aureus* to be considered as one of the most important human pathogens is largely due to its virulence potential and ubiquitous occurrence. Tissue invasion by *S. aureus* usually results from epithelial lining injury, trauma, medical or surgical interventions and viral infections [Grundmann *et al.*, 2010]. However, *S. aureus* associated food poisoning is due to ingestion of heat stable staphylococcal enterotoxins along with food [Hennekinne *et al.*, 2012]. The severity and outcome of *S. aureus* infections depend largely on the virulence of the introduced strain and the immune repertoire of the host [Grundmann *et al.*, 2010].

S. aureus strains possess a varying repertoire of virulence factors resulting in hetrogenious disease characteristics. Many of these virulence factors are encoded on MGEs [Otto, 2014] (Figure 1.1). These virulence factors mediate adherence to damaged tissue, extra-cellular

matrix and the surface of host cells; facilitate tissue destruction and spreading; bind to proteins in the bodily fluids to help evade antibody and complement-mediated immune responses; lyse host cells; and manipulate the innate and adaptive immune responses [Grumann *et al.*, 2014].

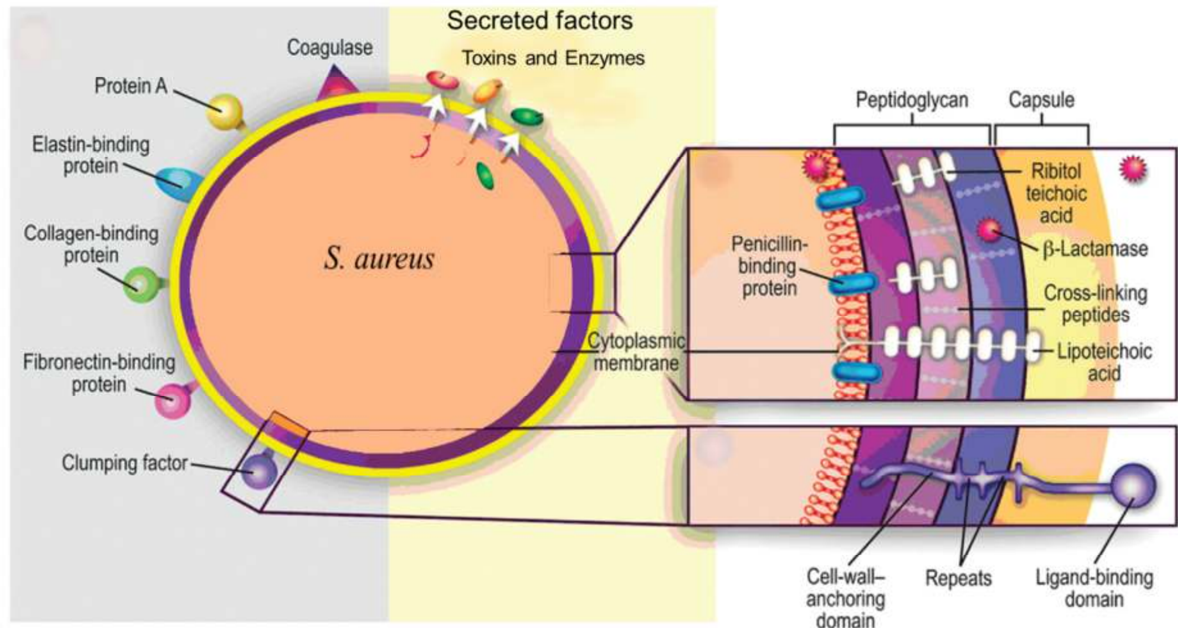


Figure 1.1: *S. aureus* virulence factors (adapted from [Gordon and Lowy, 2008])

The virulence factors of *S. aureus* are grouped in to cell surface factors, toxins and other secreted factors as discussed below.

A. Cell surface factors

The *S. aureus* surface-associated virulence factors include capsular polysaccharides (CPs), staphyloxanthin (carotenoid pigment), and a group of proteins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). About 90% of *S. aureus* clinical isolates produce polysaccharide capsule. The functions of the capsule include impeding phagocytosis by neutrophils, enhancing bacterial colonization and persistence on mucosal surfaces. The *S. aureus* golden pigment, staphyloxanthin plays an important role in resisting neutrophils' reactive oxidant mediated killing [Lin and Peterson, 2010].

The MSCRAMMs mediate attachment to different host substrates and initiate colonization leading to infection [Plata *et al.*, 2009]. The MSCRAMMs also involve in immune evasion,

bacterial aggregation and biofilms formation. As most surface proteins are encoded by the core genome [Otto, 2012], they are crucial to the success of the organism as a commensal bacterium and as a pathogen [Foster *et al.*, 2014]. The major MSCRAMMs of *S. aureus* include fibronectin-binding proteins, clumping factors, Staphylococcal protein A (Spa) and collagen-binding protein [Zecconi and Scali, 2013]. Fibronectin binding proteins A and B are important in the attachment of *S. aureus* to an extra-cellular matrix component, fibronectin and to plasma clot. Collagen binding protein is necessary for adherence of *S. aureus* to collagenous tissues and cartilage. Clumping factors A and B (ClfA and ClfB) serve as clumping and adherence of *S. aureus* cells to fibrinogen; hence, play a significant role in wound and foreign body infections [Plata *et al.*, 2009]. Staphylococcal protein A is encoded by the *spa* gene and plays mainly in immune evasion through biding with Fc region of immunoglobulin G (IgG), thus impairing opsonization and then phagocytosis [Zecconi and Scali, 2013]. Protein A is ubiquitous in *S. aureus* and often used in strain typing on the basis of variation in the DNA sequence-encoding region *X* [Foster *et al.*, 2014].

B. Toxins

S. aureus produces several toxins, some of which are found virtually in all strains, while others are restricted to a subset of strains [Otto, 2013]. The toxins can be grouped as those that can damage the membranes of host cells (pore-forming toxins), degrade inter-cellular junctions (exfoliative toxins), or modulate the immune response by aberrant activation of immune cells (Superantigen toxins) [Grumann *et al.*, 2014; Otto, 2012].

i. Pore-forming toxins

The pore-forming toxins (cytolysins) cause target cells lysis through formation of β-barrel pores in their cytoplasmic membranes [Plata *et al.*, 2009]. They include hemolysin-α, leukotoxins, and Phenol-soluble modulins (PSMs) [Krishna and Miller, 2012].

a) Hemolysin-α

Hemolysin-α (α-toxin) is encoded by the *hla* gene found in the core genome of *S. aureus* and released by about 95% of *S. aureus* strains. Binding of hemolysin-α to the membrane receptor ADAM10 (A disintegrin and metalloproteinase 10) results in the formation of

heptameric pores; thereby destroying a variety of host cells, including epithelial cells, erythrocytes, fibroblasts, monocytes, macrophages, and lymphocytes, but not neutrophils [Berube and Bubeck Wardenburg, 2013; Grumann *et al.*, 2014].

b) Leukotoxins

Leukotoxins are known to lyse cells of the myleoid lineage (i.e. monocytes, macrophages, and neutrophils) contributing to disease progression. They are either core genome- or phage-encoded, 32–35 kDa, bi-component toxins consisting of one class S and one class F protein. The class S and F proteins are non-toxic on their own, but upon oligomerization, they form a β-barreled pore-structure [Grumann *et al.*, 2014]. The leukotoxins include Panton-Valentine leukocidin (PVL), LukDE, LukAB (LukGH), LukF'M and hemolysin-γ [Otto, 2014].

Panton-Valentine Leukocidin (PVL): is prophage-encoded, bicomponent (lukF+lukS), pore-forming toxin. It is cytotoxic to neutrophils [Loffler *et al.*, 2010] and to a lesser extent to monocytes and macrophages, but not to lymphocytes. Initial binding of LukS-PV to C5a receptors on target cells triggers secondary binding of LukF-PV and subsequently induce the assembly of lytic pore-forming hetero-octamers [Spaan *et al.*, 2013]. Although PVL was first noted by Panton and Valentine in 1932 [Panton *et al.*, 1932], the interest in PVL increased enormously when an epidemiological association between presence of the *lukS* and *lukF* genes and CA-MRSA was detected [Vandenesch *et al.*, 2003]. PVL is present in about 85% of community-acquired MRSA strains, e.g. USA300/ST8; in particular those causing pneumonia, and skin and soft tissue infections, and about 5% of overall *S. aureus* clinical isolates. Despite its apparent linkage to virulent strains capable of causing deadly infections in healthy people, PVL's contribution to virulence has not been conclusively proven [Yoong and Torres, 2013]. However, a recently published meta-analysis on human staphylococcal infections found that PVL positive strains are strongly associated with skin and soft-tissue infections [Shallcross *et al.*, 2013].

LukDE and LukGH: are expressed by the majority of CA-MRSA strains similar to PVL. LukDE is present in 87% of clinical strains and is toxic toward human neutrophils and rabbit

red blood cells [Yoong and Torres, 2013]. LukGH exhibits potent cytolytic activity towards neutrophils.

LukF'M: is cytolytic to bovine polymorphonuclear leukocytes and has been implicated in bovine mastitis [Grumann *et al.*, 2014].

Hemolysin-γ: is encoded by *hlg* gene cluster of the core genome and is present in 99% of *S. aureus* strains. It is highly cytolytic to red blood cells and has moderate leukotoxic activity [Grumann et al., 2014.

c) **Phenol-soluble modulins (PSMs)**

Phenol-soluble modulins (PSMs) are a recently discovered family of amphipathic peptides with multiple roles in *S. aureus* pathogenesis. PSMs may cause lysis of many human cell types including leukocytes and erythrocytes, stimulate inflammatory responses, and contribute to biofilm development [Cheung *et al.*, 2014]. PSM peptides are encoded in the core genome with the exception of PSM*mec* which is encoded adjacent to the *mecA–mecR–mecI* gene cluster in SCC*mec* types II, III and VIII [Peschel and Otto, 2013]. PSMs are produced more abundantly by CA-MRSA compared to HA-MRSA strains [Gonzalez *et al.*, 2014].

ii. **Exfoliative Toxins**

Exfoliative Toxins (ETs) are approximately 30 kDa serine proteases which can selectively recognize and hydrolyze desmosomal cadherins in the superficial layers of the skin, i.e. keratinocytes [Bukowski *et al.*, 2010]. By loosening the keratinocyte junctions, the ETs cause blistering diseases known as bullous impetigo and staphylococcal scalded skin syndrome (SSSS). There are three known exfoliative toxins (ETs); namely ETA, ETB and ETD. The ETA is encoded by a temperate phage, ETB by plasmids and ETD by the core genome. ETA is the prevalent serotype expressed by more than 80% of ET-producing strains in Europe, USA, and Africa; however, ETB is the leading serotype in Japan [Bukowski *et al.*, 2010].

iii. Superantigen toxins

The staphylococcal superantigens (SAgs) are 20–30 kD, secreted, heat stable toxins classically associated with food poisoning and toxic shock syndrome (TSS) [Otto, 2014]. They are able to activate T-lymphocytes and antigen-presenting cells (APCs) nonspecifically by cross-linking Vβ regions of the T-cell receptor (TCR) and MHC class II molecules of the APCs in a non-antigen-specific manner [Lin and Peterson, 2010]. Formation of the complex induces intense T-cell proliferation resulting in massive cytokine production and release thereby causing capillary leak, epithelial damage and hypotension [Plata *et al.*, 2009].

Currently more than 20 distinct SAgs have been characterized from *S. aureus* strains. About half of them have been proved to be emetic, representing a potential hazard for consumers [Hennekinne *et al.*, 2012]. More than 60% of *S. aureus* clinical strains harbor at least one *sag* gene, although most strains encode many. The SAgs include the **staphylococcal enterotoxins** (SEs), **the staphylococcal enterotoxin-*like* (SE*l*s)** proteins, and **the toxic shock syndrome toxin**-1 (TSST-1) [Lin and Peterson, 2010]. SEs were originally defined by their ability to cause staphylococcal food poisoning (SFP) including emesis, and currently include the SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER, and SET. The SE*l* toxins are both homologous and structurally similar to the SEs, but either do not induce emesis or have not been formally demonstrated to induce emesis. The SE*l* toxins include the SE*l*J, SE*l*K, SE*l*L, SE*l*M, SE*l*N, SE*l*O, SE*l*P, SE*l*Q, SE*l*S, SE*l*U, SE*l*V, and SE*l*X [Xu and McCormick, 2012]. The SEs are resistant to environmental conditions (freezing, drying, heat treatment and low pH) and to proteolytic enzymes retaining their activity in the digestive tract after ingestion [Grundmann *et al.*, 2010; Ortega *et al.*, 2010].

C. Other secreted factors

More than 60% *S. aureus* clinical isolates produce bacteriophage encoded secreted proteins called chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS). The CHIPS binds specifically to the C5a and formylated peptide receptors, thereby specifically impairing the response of neutrophils and monocytes to formylated peptides and C5a [de Haas *et al.*, 2004; Postma *et al.*, 2004]. Through specifically hydrolyzing sphingomyelin; β-toxin

(sphingomyelinase C) is cytotoxic to monocytes, erythrocytes, neutrophils and lymphocytes [Lin and Peterson, 2010].

S. aureus produces two types of coagulases, staphylocoagulase and von Willebrand factor (vWF). The coagulases bind to prothrombin and several other plasma proteins, thereby triggering the conversion of fibrinogen to fibrin clots. The fibrin clots accumulate on the surface of *S. aureus* cells, inhibiting phagocytosis, causing abscess formation, and promoting adhesion of *S. aureus* to catheters during biofilm-associated infection. On the other hand, *S. aureus* produces staphylokinase which converts plasminogen to plasmin, thereby degrading the fibrin clots. This will diminish the function of the fibrin meshwork in keeping a staphylococcal infection localized. Although staphylokinase facilitates bacterial penetration through the skin barrier, it decreases the severity of skin infections by leading to drainage [Otto, 2014].

1.2.5. Clinical features of *S. aureus* infection

A. Skin and Soft Tissue Infections (SSTIs)

Skin and soft tissue infections (SSTIs) are inflammatory microbial infection of the epidermis, dermis, and subcutaneous tissues. They are common cause of hospitalization, disability, and antibiotic therapy all over the world [Ravishankar *et al.*, 2014]. The SSTIs include impetigo, cellulitis, furunculosis, subcutaneous abscesses, and infected ulcers and wounds. *S. aureus* is the leading cause of SSTIs worldwide. The SSTIs represent about 90% of all clinical manifestations of community-associated *S. aureus* disease [Cheung *et al.*, 2014]. In the hospital setting, staphylococcal skin infections are most often encountered postoperatively as surgical site infections [Tang and Stratton, 2010].

Impetigo is characterized by honey-colored crusted sores and erosions (and sometimes vesicles) on the surface of the skin [Krishna and Miller, 2012].

Cellulitis is pyogenic infection of the skin without an organized cavity and is typically limited to the epidermis, dermis, and superficial subcutaneous tissues. It is characterized by the presence of erythema, warmth, induration, and tenderness [Mistry, 2013].

Furunculosis (also called boils) is a deep infection of the hair follicle, usually by *S. aureus*, leading to abscess formation with accumulation of pus and necrotic tissue. Furuncles present as red, swollen, and tender nodules of varying size and at times with an overlying pustule. If several adjacent follicles are infected they may coalesce and form a larger nodule, known as a carbuncle [Ibler and Kromann, 2014].

Surgical site infections (SSIs) are infections involving skin, subcutaneous tissue and organs/spaces opened or manipulated during an operation, occurring within 30 days after the procedure or within one year if orthopedic implant is in situ [Mangram *et al.*, 1999].

B. Staphylococcal toxic shock syndrome (STSS)

STSS is an acute and potentially fatal disease due to a SAg-induced over production of cytokines resulting in systemic inflammation and shock in individuals lacking neutralizing antibodies to the particular SAg. It is a capillary leak syndrome stemming from toxin- and cytokine-mediated endothelium damage where patients develop fever, rash, hypotension, multi-organ involvement and convalescent desquamation [Ortega *et al.*, 2010; Xu and McCormick, 2012].

Based on the site of infection, STSS can be divided as menstrual and non-menstrual TSS. Menstrual TSS usually occurs within 2 days after the initiation of menstruation or within 2 days after completion of menstruation, and it is associated with tampon usage in women colonized vaginally by superantigen-producing *S. aureus*. TSST-1 is responsible for more than 90% of menstrual TSS. Non-menstrual TSS occurs as a complication of *S. aureus* infections after surgical procedures, burns or post-influenza pneumonia. TSST-1 is responsible for approximately half of non-menstrual TSS cases, and SEC and SEB are responsible for the majority of the remaining cases [Lin and Peterson, 2010].

C. Staphylococcal Scalded Skin Syndrome (SSSS)

Staphylococcal scalded skin syndrome, also known as Ritter's disease, is primarily characterized by skin exfoliation. Early SSSS manifests with fever, malaise, lethargy, and poor feeding. These symptoms are followed by an erythematous rash and the formation of large, fragile, fluid-filled blisters. The blisters burst with mechanical action, leaving the

affected parts of the body without a protective layer of epidermis. SSSS affects large parts of the body and the lesions are often sterile. A localized form of SSSS, restricted to the sites of infection, is recognized as "bullous impetigo" [Bukowski *et al.*, 2010].

D. Staphylococcal food poisoning

Staphylococcal food poisoning results from ingestion of staphylococcal enterotoxins (SEs). *S. aureus* can grow on different foods and secret SEs including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings [Ortega *et al.*, 2010]. Initial symptoms (nausea followed by incoercible characteristic vomiting) appear within 3 hours after ingesting contaminated food. Other commonly described symptoms are abdominal pain, diarrhea, dizziness, shivering and general weakness, sometimes associated with a moderate fever. In the majority of cases, recovery occurs within 24–48 hours without specific treatment, while diarrhea and general weakness can last 24 hours or longer [Hennekinne *et al.*, 2012].

E. Other clinical features of *S. aureus*

Besides the clinical features mentioned above, *S. aureus* is also responsible for a significant proportion of nosocomial pneumonia mainly ventilator-associated [Chastre *et al.*, 2014] and community-acquired pneumonia [Tang and Stratton, 2010]. *S. aureus* is also a leading cause of community- and hospital-acquired bacteremia. *S. aureus* bacteremia can lead to seeding of virtually any body site with infective endocarditis as a main complication [Keynan and Rubinstein, 2013]. Moreover, *S. aureus* is also responsible for an increased number of more serious infections, such as septic arthritis and osteomyelitis [Tang and Stratton, 2010].

F. Mastitis in dairy animals

Mastitis, inflammation of the mammary gland, is one of the most common and detrimental diseases cows can experience [De Vliegher *et al.*, 2012]. *S. aureus* is a major etiologic agent of mastitis in dairy farms [Cicconi-Hogan *et al.*, 2014]. It occurs when the bacteria invade the mammary gland via the teat orifice, establishing intra-mammary infection and often provoke an inflammatory response. The manifestation of mastitis can be either clinical or subclinical mastitis [De Vliegher *et al.*, 2012]. Clinical mastitis is characteristic by visible

changes in milk with appearance of flakes or blobs and with appearance of edema and pain in the udder. Subclinical mastitis is an inflammation of the mammary gland without clear signs [Varatanović *et al.*, 2010].

1.2.6. Epidemiology of Staphylococcal SSTIs in human and mastitis in cattle

The incidence of skin and soft tissue infections is increasing, concomitant with the emergence of community acquired *S. aureus*. Nowadays, SSTIs are frequently encountered in ambulatory settings such as the emergency department and primary care offices [Mistry, 2013]. In the USA alone, *S. aureus* associated skin infections account for 11.6 million outpatient and emergency room visits and nearly 500,000 hospital admissions per year [McCaig *et al.*, 2006].

A. Community onset *S. aureus* skin and soft tissue infection

Community onset *S. aureus* SSTIs are defined by the presence of culture confirmed *S. aureus* obtained within 48 hours of hospital admission or evidence of infection on admission. The community onset *S. aureus* SSTIs can be community acquired or health care-associated. They are called health care-associated community-onset (HACO) if the patient had one or more of the following in the previous year: a history of hospitalization, surgery, dialysis, or residence in a long term care facility, use of indwelling catheters or other percutaneous medical devices. However, if the criteria for HACO are absent, they are considered as community acquired [Benoit *et al.*, 2008; CDC, 2012].

S. aureus as the leading cause of skin infections in the community has been studied in several settings. For example, 75% of Skin abscesses in Mozambique [van der Meeren *et al.*, 2014] and 73.5% of skin abscesses in Sudan [El Shallaly *et al.*, 2012] are due to community onset *S. aureus.* Retrospective studies in Ethiopia documented that *S. aureus* accounted for up to 65% of isolates from community acquired wound infections [Azene and Beyene, 2011; Mulu *et al.*, 2006]. In the USA, 50-80% of community acquired *S. aureus* isolates from cutaneous abscesses are CA-MRSA [Mistry, 2013].

S. aureus transmission in the community commonly occurs through direct contact and fomites [Rodriguez *et al.*, 2014]. Risk factors associated with *S. aureus* infection in the community include overcrowding (such as in sport settings, schools, day-care centers, the military and prisons) [Graveland *et al.*, 2011], poor hygiene, and high rates of scabies, pyoderma, scratches, or insect bites [Chuang and Huang, 2013].

B. Surgical site infections

Despite advances in infection control practices, including improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis; SSIs remain a substantial cause of morbidity, prolonged hospitalization, and death [CDC, 2014]. The SSIs are responsible for 20-31% of health care–associated infections in hospitalized patients and a mortality rate of about 3% [Owens *et al.*, 2014]. SSIs also contribute to increased cost due to prolongation of hospital stay, the need for additional diagnostic tests, antibiotic treatment and, in some cases, additional surgery [Borgey *et al.*, 2012]. Infection rates among operated patients varies with hospital settings reflecting infection control practices as well as factors related to the agent, environment and the host [Seni *et al.*, 2013b]. For example, in the USA the rate of SSIs is estimated at 1.9% [CDC, 2014]. The rate is even higher in Ethiopia; for example, 8-10 % in Bahirdar [Melaku *et al.*, 2012; Mulu *et al.*, 2012], 11.4% among obstetrics cases in Jimma [Amenu *et al.*, 2011].

S. aureus is the most common organism responsible for about 30% of SSIs infection [Chiang *et al.*, 2014]. Studies from the neighbor country, Uganda, documented 20-29% of SSIs are due to *S. aureus* [Ojulong *et al.*, 2009; Seni *et al.*, 2013b]. Studies in Ethiopia also documented a significant role of *S. aureus* in SSIs. For example, *S. aureus* accounted for 28.8% of SSIs in Gondar [Kotisso and Aseffa, 1998], 26-36% in Bahirdar [Melaku *et al.*, 2012; Mulu *et al.*, 2012], 37.3% in Hawassa [Dessalegn *et al.*, 2014] and 10% in Mekele [Tesfahunegn *et al.*, 2009].

Risk factors for SSIs include, prior colonization of the incision site, host factors (such as age, nutritional status, smoking, obesity, diabetes, long-term steroid use, alcohol abuse,

malnutrition, immune-suppression and coexisting infections), the length of the preoperative hospital period, preoperative procedures (such as skin preparation/antisepsis, antimicrobial prophylaxis and preoperative shaving) and the duration and performance of the operation contribute to increased risks of SSIs [Cooper, 2013; Savage and Anderson, 2013]. Due to increase health and economic importance of SSIs, evidence based guidelines on prevention of SSIs has been published [Berrios-Torres *et al.*, 2017].

C. Mastitis in dairy cattle

Mastitis is one of the most common and detrimental diseases cows can experience [De Vliegher *et al.*, 2012]. It is the leading cause of economic losses in dairy cattle herds, because of poor yields in the infected udder, veterinary treatments, milk that must be discarded [Peton and Le Loir, 2014]. Besides the economic impact, mastitis is also an important animal health and welfare issue [Keefe, 2012].

S. aureus is one of the leading etiologies of mastitis in dairy animals. In addition to the economic and animal health impacts, mastitis due to *S. aureus* is also a potential public health problem as many isolates of milk from infected glands possess enterotoxin genes [Peton and Le Loir, 2014]. *S. aureus* transmission in dairy cattle is thought to occur primarily via the milking machine, udder cloths or milkers' hands [Zadoks *et al.*, 2011]. Most infections are established during the first three months of lactation, and approximately 80% of them persist until the end of lactation. If no antibiotic therapy is administered during the dry period, most of these infections will still be present after calving [Peton and Le Loir, 2014].

S. aureus is the most common udder pathogen in bovine mastitis in Sweden accounted for 21.3% of acute clinical mastitis [Ericsson Unnerstad *et al.*, 2009] and 19% of subclinical mastitis [Persson *et al.*, 2011]. In Ethiopia, *S. aureus* accounted for 47% of bovine mastitis etiologies in Wondogenet [Abdella, 1996], 42.6% in Selalle area [Getahun *et al.*, 2008], 19% in Bahirdar [Almaw *et al.*, 2008], 48.6% around Hawassa [Abera *et al.*, 2012], 36% in Northern Ethiopia [Haftu *et al.*, 2012] and 16.2% around Addis Ababa [Mekuria *et al.*, 2013].

D. Zoonosis

Most strains of *S. aureus* are host-specific, indicating a low frequency of cross-species transmission. However, recent studies have identified several *S. aureus* strains that are associated with multiple host species, implying either zoonotic transmission or a recent common ancestor [Spoor *et al.*, 2013]. Of particular concern is the zoonotic transmission of MRSA that has occurred in Europe over the last decade [Smith *et al.*, 2014]. Transmission of *S. aureus* from animals to humans can take place either by direct contact or by contact with excretions of the animals [Wendlandt *et al.*, 2013], environmental contamination, and eating or handling contaminated meat [Verkade and Kluytmans, 2014] and dairy products [Doyle *et al.*, 2012].

The first zoonotic MRSA, so called livestock-associated MRSA (LA-MRSA), was first reported in humans in the Netherlands in 2003 [van Loo *et al.*, 2007; Voss *et al.*, 2005]. Since then LA-MRSA has increased to 32% in 2006 [van Rijen *et al.*, 2008] and 42% by the end of 2008 among all newly identified MRSA strains in humans in the Netherlands. The main risk groups for MRSA CC398 carriage are humans with occupational exposure to pigs and veal-calves. LA-MRSA is rarely found outside these risk groups [Verkade and Kluytmans, 2014]. LA-MRSA is also reported from other European countries [Armand-Lefevre *et al.*, 2005; Cuny *et al.*, 2009; Espinosa-Gongora *et al.*, 2012; Monaco *et al.*, 2013; Porrero *et al.*, 2012], North America [Dressler *et al.*, 2012; Frana *et al.*, 2013], and Asia [Asai *et al.*, 2012; Wagenaar *et al.*, 2009]. The predominant LA-MRSA in Europe and North America is ST398; however, ST9 predominates in Asia [Asai *et al.*, 2012; Fang *et al.*, 2014].

Another form of MRSA harboring *mec*C was isolated recently from animals and later from human during retrospective investigation in the UK and Denmark [Garcia-Alvarez *et al.*, 2011]. The *mec*C positive MRSA isolates were found to harbor type XI SCC*mec* and belonged predominantly to CC130 and ST425. These *mec*C MRSA isolates have also been reported from other European countries, including Republic of Ireland, Germany, France, Netherlands, Belgium, Sweden, Norway, Austria, Spain, Switzerland and Finland [Paterson *et al.*, 2014]. Other *S. aureus* molecular types capable of colonizing both human and cattle

include ST1, ST8, and CC97 [Fitzgerald, 2012]. The CC97 is the leading cause of bovine mastitis in Europe, Asia, and North and South America [Spoor *et al.*, 2013].

1.2.7. Laboratory Methods for diagnosis of *S. aureus* Infection

A. Microscopy

When observed microscopically in Gram stained smear, *S. aureus* appears as a gram-positive coccus arranged in grape-like clusters as shown in Figure 1.2.

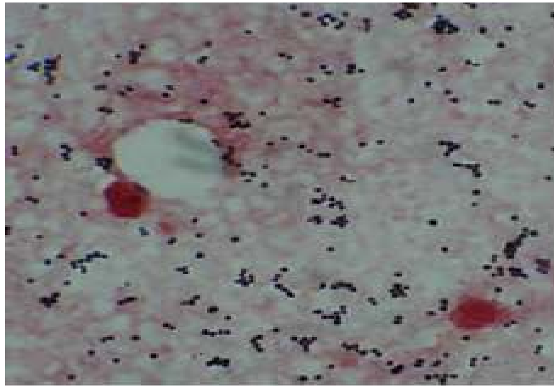


Figure 1.2: Gram stain from tissue with *S. aureus* infection [Tang and Stratton, 2010]

B. Culture

Currently, culture is the most commonly used method for the diagnosis of *S. aureus* infection. *S. aureus* grows readily on several types of culture media. When grown in an enriched media, such as sheep blood agar, it usually produces characteristic β-hemolytic golden color colonies [Tang and Stratton, 2010]. *S. aureus* grows best when incubated aerobically at a temperature of 35^0C. Moreover, it is able to grow at high NaCl concentration and is capable of fermenting mannitol. Hence, mannitol salt agar containing 7.5% NaCl has been used as a selective medium [Missiakas and Schneewind, 2013]. Besides the above mentioned characteristics; *S. aureus* is catalase positive, coagulase positive and Deoxyribonuclease (DNase) positive. These characteristics are often used during phenotypic identification of *S. aureus* [Brown *et al.*, 2005].

C. Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing is performed for the rapid and reliable prediction of antimicrobial success in the treatment of infection. AST for *S. aureus* can be performed by

phenotypic or nucleic acid-based methods [van Belkum and Dunne, 2013]. The phenotypic AST methods are the most widely used methods that include the classical disc diffusion method, broth microdilution, antimicrobial gradient methods (e.g. E-test strips), and various commercially available automated systems (e.g. the Vitek 2 system from BioMérieux). These methods have high sensitivity for detecting antibiotic resistance, have been highly standardized; such as those published by CLSI and EUCAST [Pulido *et al.*, 2013]. Nucleic acid-based AST methods detect resistance genes by PCR methods. For example, methicillin resistance by *S. aureus* can be determined by the resistance genes (*mecA/mecC*) using multiplex PCR [Becker *et al.*, 2013]. However, the nucleic acid methods cannot detect all resistance markers, are expensive, and have not been widely adopted [van Belkum and Dunne, 2013].

D. Molecular Methods

Most molecular methods for identification of *S. aureus* have been PCR based. A range of primers designed to amplify species-specific targets have now been developed. Such targets include the nuclease (*nuc:* frequently used), coagulase (*coa*), protein A (*spa*), and *16S rRNA* [Brown *et al.*, 2005]. The diversity of *S. aureus* infections and virulence determinants warrant typing methods to identify strains with unusual pathogenic abilities, to investigate the source and route of spread of infection, and for the formulation of effective control measures. Over the years, many *S. aureus* molecular typing methods, including pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *S. aureus* protein A (*spa*) typing and SCC*mec* typing for MRSA have been used [Udo, 2013]. The *spa* typing and SCC*mec* typing are specific to *Staphylococci* [Mediavilla *et al.*, 2012].

These molecular techniques have been used successfully to describe the global population structure of *S. aureus*, to study their subsequent worldwide dissemination, to study the evolution of the MRSA clones, to provide a framework for the description of the major lineages associated with healthcare-associated infections in different countries, and to monitor their emergence, dispersal and decline in different settings [Price *et al.*, 2013].

I. Pulsed-field gel electrophoresis (PFGE)

PFGE is a classic 'fingerprinting' method which compares banding patterns from whole-genome macro-restriction digests, and is considered the gold standard for outbreak investigations involving closely-related strains [Mediavilla *et al.*, 2012]. In PFGE for *S. aureus*, the chromosomal DNA is digested with the restriction enzyme *Sma*I, and the resulting DNA fragments are separated by agarose gel electrophoresis in an electric field with an alternating voltage gradient. The resulting banding patterns are analyzed using special software packages, such as GelCompar II from Applied Maths, using Dice comparison and unweighted pair group matching analysis (UPGMA) settings [Deurenberg and Stobberingh, 2008] according to the criteria of [Tenover *et al.*, 1995].

Although highly discriminating, inter-laboratory comparisons and data portability are limited due to the lack of internationally harmonized PFGE protocols, such that similar strains may reflect national or regional nomenclature. The PFGE database in the United States, for example, classifies major *S. aureus* clones as USA100, USA200, USA300, and so on, whereas other international designations include EMRSA (United Kingdom), WA (Western Australia), and CMRSA (Canada) [Mediavilla *et al.*, 2012].

II. Multi-Locus Sequence Typing (MLST)

MLST has been proven to be an excellent method to study the molecular evolution of *S. aureus*. This method is based on the sequence analysis of fragments of seven *S. aureus* housekeeping genes, i.e. *arc*C*, aro*E*, glp*F*, gmk, pta, tpi and yqi*L, each approximately 500-bp in length [Deurenberg and Stobberingh, 2008]. The fragments are sequenced and submitted to the *S. aureus* MLST database for identification (http://s*aureus*.mlst.net). Strains with identical sequences at all seven genetic loci are assigned unique 'sequence types' (ST), and clusters of closely related STs are called 'clonal complexes, CCs'. MLST provides excellent inter-laboratory reproducibility and data portability. However, MLST in most cases will be of limited use for tracking the spread of individual *S. aureus* clones due to the insufficient discriminatory power of the method. Even though a large number of STs has been discovered, a limited number of clonal complexes (CC1, 5, 8, 15, 22, 30, 45, 59, 80, 97, and 121) appear to predominate the *S. aureus* population. These clonal complexes

display a worldwide distribution, as they have been found ubiquitously through local and national surveys in many countries on every continent [Nubel *et al.*, 2011].

Outbreaks of CA-MRSA infections have been reported worldwide and successful clones are usually associated with specific geographical locations. Clones with ST8 are mostly reported in the USA and Canada, with ST80 in Europe, ST59 in the Asia-Pacific region, and ST30 worldwide [Chuang and Huang, 2013]. ST80 is also reported as the predominant type in the North African country, Tunisia [Mariem *et al.*, 2013]. However, ST152 followed by ST15 and ST121 are the most frequent types in Ghana [Egyir *et al.*, 2014].

III. *spa* typing

spa typing is a single locus nucleotide sequence-based method that targets the polymorphic variable-number tandem repeat (VNTR) region of staphylococcal protein A (*spa*). The *spa* repeats are typically 24 bp in length, with presumptive duplications, deletions, and rearrangements contributing to the identification of more than 10, 000 unique patterns known as '*spa* types' [Mediavilla *et al.*, 2012]. The *spa* typing method involves sequencing of the 24-bp variable repeat unit in the 3′ region of the protein A gene (*spa*) [Udo, 2013] and comparing the pattern of repeats to a public databases (http://spaserver.ridom.de). So far, as the technique is straightforward and cost-effective, it is noteworthy that the clonal background predicted by *spa* typing usually agrees with that of MLST, and can therefore be used to infer clonal complexes (CC). As with MLST, *spa* typing data is unambiguous and portable [Mediavilla *et al.*, 2012].

The *spa* type 70 predominates in Tunisia [Mariem *et al.*, 2013]. However, t355 followed by t084 and t314 are the most frequent types in Ghana [Egyir *et al.*, 2014]. In, Uganda the predominant *spa* lineages were t645 followed t4353 [Seni *et al.*, 2013a].

IV. SCC*mec* typing

Unlike PFGE, MLST, and *spa* typing which describe the genetic background of *S. aureus*, SCC*mec* typing classifies distinct allotypes of SCC*mec* elements present in MRSA strains [Mediavilla *et al.*, 2012]. Eleven SCC*mec* types (I–XI) based on complete nucleotide

sequence data have been described to date in *S. aureus* that range in size from 20 to 60 kb. The majority of MRSA described to date harbor *mecA* and are designated SCC*mec* types I–X. The recently identified Livestock-associated (LA)-MRSA harboring *mecC* is designated SCC*mec* type XI [Shore and Coleman, 2013].The frequently encountered CA-MRSA strains are typically SCC *mec* IV, V, and VI, while HA-MRSA ones are usually SCC *mec* I, II, and III [Udo, 2013]. The SCC*mec* types I to III are relatively big in size and carry multiple antibiotic resistance determinants. Types IV and V were recognized as new versions of SCC*mec* that were almost diagnostically harbored by CA-MRSA [Hiramatsu *et al.*, 2013].

Contemporary conventions for MRSA nomenclature rely on the combination of MLST sequence type (or clonal complex) and SCC*mec* type (e.g. ST5-II, ST8-IV), with additional resolution provided by other molecular methods such as *spa* typing. SCC*mec* subtyping can provide additional information, often discriminating between distinct lineages within a given clonal background, as in the case of the North American (ST8-IVa) and Latin American (ST8-IVc) lineages of USA300. This approach, while not always exhaustive or unambiguous, allows for a universal short-hand to catalog the regional and historic clone names which appear frequently in the literature [Mediavilla *et al.*, 2012].

1.2.8. Treatment of *S. aureus* infections

Antibiotic therapy for *S. aureus* infections are better guided by susceptibility testing due to the increased resistance to several antibiotics [Keynan and Rubinstein, 2013]. Simple abscesses or boils are usually managed by incision and drainage. However, antibiotic therapy is recommended for abscesses associated with severe or extensive disease (e.g., involving multiple sites of infection) or rapid progression in presence of associated cellulitis, signs and symptoms of systemic illness, associated co-morbidities or immune-suppression, extremes of age, abscess in an area difficult to drain (e.g., face, hand, and genitalia), and lack of response to incision and drainage alone [Ibler and Kromann, 2014]. For methicillin susceptible *S. aureus* (MSSA) infections, β-lactams (such as nafcillin, oxacillin and cefazolin) are used as the first-line therapy [Li *et al.*, 2017; Lin and Peterson, 2010].

The clinical practice guideline by the Infectious Diseases Society of America (IDSA) recommends the following for the treatment of MRSA infections. For empirical coverage of CA-MRSA in outpatients with SSTI, oral antibiotic options include clindamycin, trimethoprim-sulfamethoxazole, tetracyclines (doxycycline or minocycline), and linezolid. For hospitalized patients with complicated SSTI, options include intravenous (IV) vancomycin, oral (PO) or IV linezolid, daptomycin IV, telavancin IV, and clindamycin IV or PO. However, due to increase in drug resistance, antibiotic therapy should be guided by susceptibility testing result. Seven to 14 days of therapy is recommended but should be individualized on the basis of the patient's clinical response [Liu *et al.*, 2011].

1.2.9. Drug resistance in *S. aureus*

Anti-staphylococcal antibiotics target multiple pathways including bacterial cell-wall synthesis (i.e. β-lactams, glycopeptides), folic acid metabolism (sulfonamides), and bacterial protein synthesis (i.e., macrolides, lincosamides and aminoglycosides). However, *S. aureus* is smart enough in developing resistance to many of the antibiotics to ensure its survival [Lin and Peterson, 2010].

A. Resistance to β-Lactams

The β-Lactams bind to the penicillin-binding proteins (PBP) essential for cell wall biosynthesis and inhibit peptidoglycan crosslink formation, leading to bacterial cell lysis [Paterson *et al.*, 2014]. Resistance to β-lactams in *S. aureus* results mainly either through enzymatic inactivation by β-lactamase or target site replacement by the gene products of the *mecA* or *mecC* [Wendlandt *et al.*, 2013].

In the early 1940s, prior to the introduction of penicillin, the mortality rate of individuals with *S. aureus* systemic infection was about 80% [Skinner and Keefer, 1941]. Soon after, the introduction of penicillin in 1941 has saved the lives of many patients. However, 2 years later, the first penicillin resistant *S. aureus* was observed in a hospital and later in the community [Deurenberg and Stobberingh, 2008] due to the acquisition of a plasmid coding for penicillinase (β-lactamase) [Turlej *et al.*, 2011]. The β-lactamase hydrolyzes the β-lactam ring of penicillin thereby inactivating the antibiotic. It is encoded by the *bla*Z gene

and the closely linked regulatory genes, *bla*I and *bla*R. Although initially highly effective for the treatment of *S. aureus* infections, today over 90% of human *S. aureus* strains are resistant to penicillin [Malachowa and DeLeo, 2010]. Indeed, 100% *S. aureus* resistance to penicillin has been documented in a recent study done in Jimma, Ethiopia [Kejela and Bacha, 2013].

To overcome infections caused by β-lactamase-producing *S. aureus*; β-lactamase-resistant, narrow spectrum, semi-synthetic penicillin (i.e. methicillin) was introduced in 1959 [Turlej *et al.*, 2011; Waness, 2010]. The victory against *S. aureus* using methicillin was short-lived with the first methicillin-resistant *S. aureus* (MRSA) strain identified in 1961 [Barber, 1961]. Resistance to β-lactams in MRSA is conferred by the acquisition of a mobile genetic element, the staphylococcal cassette chromosome (SCC*mec*) carrying the *mecA* gene or the recently discovered *mec*C. Both *mec* genes code for alternative penicillin-binding proteins with a strongly reduced affinity to virtually all β-lactam antibiotics [Wendlandt *et al.*, 2013]. Shortly thereafter, MRSA became pandemic in many medical institutions worldwide [Waness, 2010].

Later on, community acquired MRSA was reported from populations lacking risk factors for exposure to the health care systems from western Australia in 1993 [Udo *et al.*, 1993]. Since the first report of community acquired MRSA, there has been an explosion in the number of CA-MRSA infections worldwide [David and Daum, 2010]. Indeed, CA-MRSA strains are now spreading in the healthcare facilities, displacing in some countries typical HA-MRSA [Mediavilla *et al.*, 2012; Turlej *et al.*, 2011]. For example, in Canada, >20% of MRSA infections in the healthcare setting can be attributed to community acquired strains [Nichol *et al.*, 2013].

CA-MRSA most commonly manifests as skin and soft tissue infections, although invasive disease such as sepsis and necrotizing pneumonia can occur. Unlike HA-MRSA, CA-MRSA typically affect those who lack established risk factors for the acquisition of MRSA. CA-MRSA also differs from HA-MRSA in that they are generally more susceptible to a variety of non-β-lactam antimicrobial agents. In addition, the majority of CA-MRSA strains harbor

virulence determinants such as the PVL as well as other toxins that may contribute to the increasing morbidity and mortality associated with CA-MRSA infections [Chuang and Huang, 2013; Nichol *et al.*, 2013].

Although the first methicillin-resistant *S. aureus* (MRSA) strain was identified in 1961 from human [Barber, 1961] it has been rarely isolated from livestock till recently. Nowadays, it has been reported with increased frequency from a wide variety of dairy animals, including cows [Fitzgerald, 2012]. A study from Brazil reported MRSA from 11% of dairy cows with mastitis [Silva *et al.*, 2014]. Among *S. aureus* strains isolated from bovine milk with mastitis in Turkey, 17% were resistant to methicillin [Turkyilmaz *et al.*, 2010]. In another study from Belgium, 9.3% of *S. aureus* isolates from mastitis milk samples were MRSA [Vanderhaeghen *et al.*, 2010]. In Korea, 6.2% of *S. aureus* isolates from bovine mastitis milk were MRSA [Nam *et al.*, 2011]. A study in smallholder dairy farms in Ismailia, Egypt reported 52% of *S. aureus* isolates from dairy cows' milk were MRSA [Elhaig and Selim, 2014]. In Ethiopia there have been few studies conducted addressing MRSA in dairy cows [Mekuria *et al.*, 2013; Tigabu *et al.*, 2015] and none of them found *mec*A.

B. Resistance to Vancomycin

Vancomycin inhibits bacterial cell wall biosynthesis by forming complexes with the D-ala-D-ala portion of peptide precursor unit to prevent the cross-linking of the cell wall peptidoglycan [Lin and Peterson, 2010]. Resistance to Vancomycin can be grouped as intermediate (VISA) and high (VRSA). Intermediate resistance is more common than high resistance and results from thickening of the *S. aureus* cell wall. The excess D-ala-D-ala targets in the cell wall act as a molecular sink, impairing vancomycin from accessing its target. Vancomycin resistance in VRSA results most likely from horizontal transfer of Tn*1546* carrying the *van*A operon to *S. aureus* from vancomycin-resistant enterococci (VRE) during co-infection [Deresinski, 2013].

Vancomycin was approved by the U.S drug and food administration (FDA) in 1958 [Waness, 2010] and became the drug of choice for patients infected with MRSA [Cole and Riordan, 2013]. However, in 1997 the first case of vancomycin-intermediate *S. aureus*

(VISA) was reported from Japan [Hiramatsu *et al.*, 1997] and vancomycin-resistant *Staphylococcus aureus* (VRSA) in 2002 from USA [Sievert *et al.*, 2008]. VRSA is also reported outside the USA, including from India [Thati *et al.*, 2011], Iran [Azimian *et al.*, 2012] and Pakistan [Hakim *et al.*, 2007].

1.2.10. Prevention and control of *S. aureus* infections

Keeping personal hygiene and appropriate wound care are recommended for patients with skin and soft tissue infections (SSTI). Wounds should be covered with clean, dry bandages and good personal hygiene with regular bathing and washing of hands with soap and water, or cleansing with an alcohol-based hand gel is recommended [Ibler and Kromann, 2014]. Preventive measures for SSIs target at reducing opportunities for microbial contamination of the patient's tissues or sterile surgical instruments [Mangram *et al.*, 1999]. The tenets of mastitis prevention are maintaining cows in a clean, dry, comfortable environment and ensuring that recommended milking practices are consistently followed [Erskine, 2012]. Currently, there is no approved vaccine or immunotherapy to prevent or treat *S. aureus* diseases [Lin and Peterson, 2010].

The best measures to prevent enterotoxin associated food poisoning in *S. aureus* rely on preventing the bacteria from contaminating food and discourage to continue replication. This is because Staphylococcal enterotoxins are stable to heat treatment and stomach acid [Schelin *et al.*, 2017]. Practicing hand hygiene measures are important to avoid or reduce contamination of food by *S. aureus*. These procedures must include control of raw materials, proper handling, cleaning and disinfection of equipment from farm to fork. However, as these requirements are usually not sufficient, it is necessary to destroy *Staphylococci* through appropriate treatment, thermal or otherwise, to prevent their growth under refrigerated conditions. Using cold chain is critical in regard to *Staphylococci* especially for foods served at large gatherings such as social events [Hennekinne *et al.*, 2012].

1.3. Statement of the problem

Staphylococcus aureus is an unusually successful and adaptive pathogen that can cause diverse array of diseases despite its frequent carriage as a commensal [Ortega *et al.*, 2010].

It was a major cause of morbidity and mortality before the advent of antibiotics and still continues to be one of the major threats to the public health [Juhas, 2013]. This stems from the ability of the bacterium to colonize diverse hosts, possession of wide range of virulence factors responsible to diverse diseases, and the continuous evolution of resistance to many of the available antibiotics [Ortega *et al.*, 2010]. Despite its importance and the large number of reports from developed countries; there is little information in the developing world, not because of low prevalence but probably because of under-reporting and scarce diagnostic microbiology facilities [Chuang and Huang, 2013].

The clinical relevance of *S. aureus* is increasing due to the increasing prevalence of antibiotic resistant strains, more importantly MRSA, both in the health care settings and in the community [Zecconi and Scali, 2013]. Although MRSA initially emerged in the hospital settings and caused epidemics of hospital acquired infections, more virulent strains have emerged among previously healthy individuals in the community without links to healthcare settings in the 1990s. Nowadays, these more virulent CA-MRSA strains are increasingly reported even from hospital settings [Nichol *et al.*, 2013; Paterson *et al.*, 2014]. Although MRSA is nowadays well documented in many parts of the world, data concerning MRSA is limited in sub-Saharan African countries like Ethiopia [van der Meeren *et al.*, 2014].

Livestock acquired (LA) MRSA, which has spread extensively in livestock animals, has emerged recently among high risk groups who have occupational contact with these animals [Verkade and Kluytmans, 2014]. The emergence of LA-MRSA is not only important from an animal welfare and economic perspective but can act as a reservoir for zoonotic infection of humans [Paterson *et al.*, 2014]. Although, LA-MRSA is well documented in North America, Europe and Asia; to our knowledge it has never been assessed in Ethiopia. Assessment of LA-MRSA in Ethiopia is important as Ethiopia is the leading livestock producing country in Africa and among the 10 leading countries in the world. Indeed, most peoples in the rural areas of the country have the habit of living in close contact with different domestic animals, including bovine and raw milk consumption is common.

S. aureus has also developed resistance even to Vancomycin, the drug of choice for the treatment of MRSA, and to other multiple antibiotics, including macrolides, aminoglycosides and fluoroquinolones [Marasa *et al.*, 2014]. The development of resistance both in human and animals has been associated with the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production [Ortega *et al.*, 2010]. This leads to the selection, transmission, and persistence of multidrug resistance traits in *S. aureus* [Marasa *et al.*, 2014]. The problem could be even worse in the resource limited countries, such as Ethiopia, where treatment is mainly empirical due to a lack of appropriate laboratory facilities.

Molecular typing of *S. aureus* allows several important observations to be made regarding the evolution, epidemiology, and spread of clones with particular public health importance, such as HA-MRSA, CA-MRSA, and LA-MRSA. For MRSA, surveillance is particularly important because it appears that certain clones have disseminated over wide geographical regions. Moreover, little is known about the population structure and geographical abundance of MSSA, which provides the genetic reservoir from which MRSA emerge [Grundmann *et al.*, 2010]. Not unexpectedly, molecular based studies are limited in the resource limited parts of the world, like Africa. In Ethiopia only few studies addressing the molecular epidemiology of *S. aureus* were done [Seyoum *et al.*, 2016; Tarekgne *et al.*, 2016; Tigabu *et al.*, 2015]. But all of these studies were done on milk isolates.

1.4. Significance of the study

The present study provides in depth information on the drug resistance pattern, virulence gene profile and genotypes of *S. aureus* isolates from human patients with skin and soft tissue infections, nares of dairy farmers and milk of dairy cows in Mekele, Northern Ethiopia.

Up-to-date information on the burden of skin and soft tissue infections of human and intramammary infections of dairy cows due to *S. aureus* and its drug resistance pattern is important to consider the best possible infection prevention measures and guide the empiric therapy of the above mentioned diseases in the study area. *S. aureus* isolates, especially milk

isolates, with the capacity to produce enterotoxins can pose a risk of food poisoning and toxic shock syndrome to the public. Hence, exploring the toxin profile would help to design and implement preventive measures to reduce hazards from unsafe milk consumption.

Genotyping of human and animal *S. aureus* isolates could help to understand the genotypic diversity of the isolates, and whether unique clones are adapted to the study area or are similar with those clones spread globally. In addition, the genotyping data could highlight whether there are *S. aureus* types which are adapted to both human and animals indicating cross-transmission between them. This in turn could be important to consider the one health concept for better infection prevention and control measures and prevent further transmission.

To the best of our knowledge, this study is the first in Ethiopia in studying *S. aureus* isolates from both human and dairy cows; hence, it can serve as a baseline study for further related studies to be conducted in the Country.

1.5. Hypothesis

- *S. aureus* isolates from human SSTIs carry more frequently the toxin genes *pvl* and *tsst-1* than dairy farm isolates
- There is difference on *S. aureus* molecular types circulating in the study area from those reported elsewhere
- There is clonal spread of specific *S. aureus* strains in the study area
- There is cross-transmission of *S. aureus* between human and dairy cows in the study area

1.6. OBJECTIVES OF THE STUDY

1.6.1. General Objective

- To determine the phenotypic and molecular characteristics of *S. aureus* isolates from humans and animals in Mekele city, Northern Ethiopia

1.6.2. Specific Objectives

- To isolate *S. aureus* from skin and soft tissue infections of human, milk of dairy cows and nares of farm workers
- To determine the antibiotic susceptibility pattern of the *S. aureus* isolates to selected antimicrobial agents using disk diffusion method and E-test
- To characterize the major virulence and drug resistance genes of the *S. aureus* isolates using PCR
- To determine the genotypic diversity and clonal relatedness of *S. aureus* isolates from human and dairy cows

CHAPTER TWO: METHODS AND MATERIALS

2.1. Study area

This study was carried out in Mekele, the capital city of the Tigray region. Tigray is located in Northern Ethiopia and is divided in to 6 zones (southern, south eastern, eastern, central, western, and north western) and one special zone (Mekele city) (Figure 2.1). Based on the 2007 census, the total human population of the region was around 4.3 million. According to the 2017 report by the central statistical agency of Ethiopia, the cattle population of the region was around 4.8 million. Among it around 2.4 million (51%) were females and 28,133 were dairy cows (for milk purpose) [CSA, 2017].

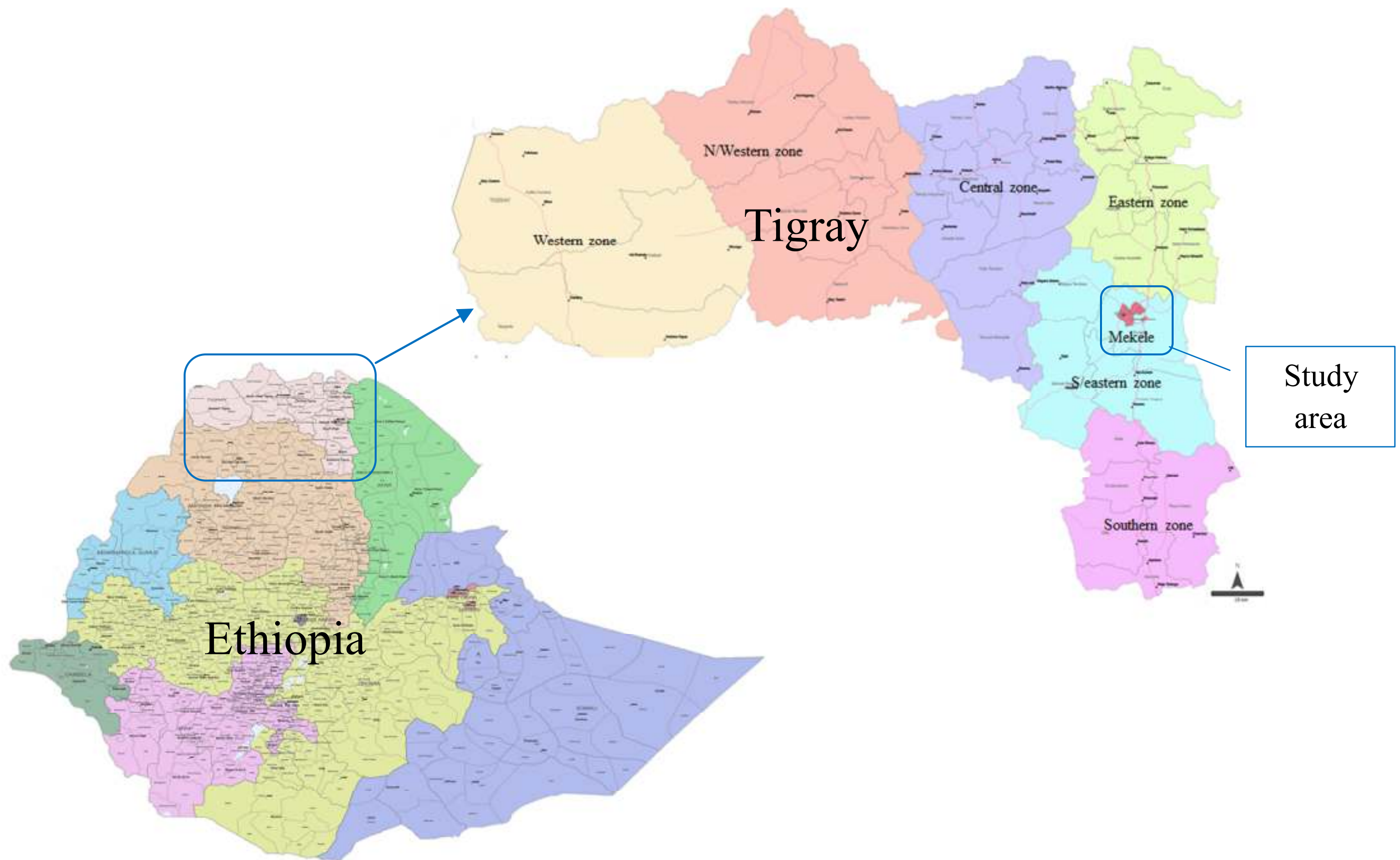


Figure 2.1: Map of Ethiopia: Tigray region administrative map (adapted from UN Office for the Coordination of Humanitarian Affairs, OCHA website (published on 17 Aug 2017) *https://www.humanitarianresponse.info/sites/www.humanitarianresponse.info/files/documents/files/21_adm_eth_081517_a0.pdf*)

Mekele is located 783 kilometers north of Addis Ababa. There are seven hospitals in the city including, one referral hospital, 3 general governmental public hospitals, one defense

hospital, and three private hospitals [Hagos *et al.*, 2014]. The current study on skin and soft tissue infections was conducted in Ayder Referral Hospital. This hospital is the only referral hospital in the city and the region in general. It stands as the second largest hospital in Ethiopia with the total capacity of about 500 inpatient beds and an estimated patient flow of more than 100,000 per year. The hospital also serves for parts of Amhara and Afara regional states. (http://www.mu.edu.et/index.php/the-ayder-referral-hospital).

The dairy cows and farm workers were selected from a total of 67 Small and large scale dairy farms located in 3 sub-cities of Mekele; namely Hawolti, Semen, and Hadnet.

2.2. Study design and period

A prospective cross sectional study was conducted from March, 2016 to August, 2017 to characterize *S. aureus* isolates from humans and dair cows.

2.3. Study Population

Humans

- Patients of all age groups with suspected skin and soft tissue infections (both community and health care associated) who attended Ayder referral hospital for investigation and management.
- Dairy farmers working at the dairy farms where the cows were selected in Mekele

Animals

- Lactating dairy cows from dairy farms in Mekele were included in the study.

2.4. Sample size and sampling technique

The sample sizes for each group were calculated using the single population proportion formula based on prevalence of *S. aureus* among patients with pus and/or wound discharge (SSTIs) at Gondar university hospital, 23% [Muluye *et al.*, 2014] and prevalence of *S. aureus* among lactating cows in and around Mekele,50%, confidence level of 95% and 5% significance level (precision).

$$n=\frac{Z^2P(1-P)}{d^2}$$

Where n is sample size, Z is the standard normal variate corresponding to 95% confidence interval (1.96), P is the expected prevalence mentioned above for each group, and d is the required precision of the estimate (5%).

Therefore, the following sample sizes were considered in this study

- **355** study participants with SSTIs (community onset SSTIs and SSIs)
- **385** lactating dairy cows
- **71** dairy farm workers in the dairy farms

All human study participants visiting Ayder referral hospital with skin and soft tissue infections (SSTIs) that fulfill the inclusion criteria were conveniently included until the required sample size was met.

For the dairy farms, first list of dairy farms and number of cows in lactational stage were identified from the city's agriculture bureau. Based on this information, all the lactating cows and all the available dairy farm workers during data/sample collection were included.

I. Inclusion criteria

Human study participants with SSTIs

- Patients of all age groups with suspected community-onset SSTIs visiting Ayder referral hospital and willing to participate in the study.
- Patients of all age groups with clinically suspected SSIs within 30 days of the surgical procedure or within one year if orthopedic implant was in situ and willing to participate in the study

Dairy cows

- Lactating cows from small or large scale dairy farms in Mekele where farm owners/keepers/administrators allowed their cows to be included in the study.

Dairy farmers

- All available and consenting workers from the dairy farms were included

II. Exclusion criteria

- Potential participants (patients with suspected community-onset SSTIs, dairy cows and farm workers) who took antibiotics in the past two weeks

- Patients with SSIs where their wounds were cleaned, disinfected and dressed before sample collection

2.5. Operational Definitions

***S. aureus* associated Community Onset Skin and Soft tissue infections (CO-SSTIs):** are defined by the presence of culture confirmed *S. aureus* obtained within 48 hours of hospital admission/visit or evidence of infection on admission.

Community acquired *S. aureus* infection: the presence of culture confirmed *S. aureus* obtained within 48 hours of hospital admission/visit or evidence of infection on admission and the patient did not have one or more of the following in the previous year: a history of hospitalization, surgery, dialysis, or residence in a long term care facility, use of indwelling catheters or other percutaneous medical devices [Wang *et al.*, 2015].

Surgical Site infection (SSI): clinically apparent infections of post-operative wounds that develop within 30 days of the procedure or within one year if orthopedic implant was in situ [Mangram *et al.*, 1999].

Multi Drug Resistant (MDR) *S. aureus*: *S. aureus* isolate resistant to three or more classes of antimicrobials recommended for *S. aureus* treatment [Magiorakos *et al.*, 2012]

2.6. Data collection

Study participants with CO-SSTIs: Data regarding socio-demographic characteristics (e.g. sex, age, residence etc) was collected using a structured data collection form by trained nurses (Annex III-A). Samples from community onset-SSTIs were labeled as CO-001, CO-002, CO-003….CO-217.

Study participants with SSIs: information regarding date of infection, ward, hospital stay etc. was collected in addition to the socio-demographic characteristics by trained nurses (Annex III-B). Samples from surgical site infection (SSI) were labeled as S-001, S-002, S-003…S-138.

Dairy farm workers: Socio-demographic data, specific duty in the farm etc. were collected using a structured data collection form by professionals in veterinary medicine (Annex III-C). Nasal swab (NS) samples from farm workers were labeled as N-01, N-02, N-03…N-71.

Dairy cows: Data regarding dairy farm**,** age, parity, lactational stage, clinical findings etc. were collected using a structured data collection form by veterinary medicine professionals (Annex III-D). Milk samples from dairy cows were labeled C-001, C-002, C-003 …C-384.

2.7. Specimen Collection, Handling and Transport

A. Wound specimen

Wound swab was collected from informed and consented study participants (Annex I-A, I-C, I-D and Annex II-A, II-C, II-D) with suspected SSIs and those with community onset open wound infection by trained nurses following Levine's technique [Levine *et al.*, 1976] using BD culture swab and collection system containing Amies transport media (Becton Dickinson and Co., USA). Before collection, the wound was cleansed using normal saline and sterile gauze. Then, wound specimens were collected by rotating the sterile culture swab over a 1 cm^2 area of the viable wound tissue with sufficient pressure to extract fluid from within the wound tissue. The culture swab was then returned back to its tube, labeled and transported to the laboratory immediately.

B. Pus from Impetigo/Boils/Folliculitis/Carbuncle

After disinfecting the collection area using 70% alcohol, pus samples were collected from informed and consented study participants (Annex I-A, I-C, I-D and II-A, II-C, II-D) by aspiration or directly collecting from a drainage tube if an abscess was incised and drained for medical intervention. When pus was not enough to be aspirated, pus swab was collected using BD culture swab and collection system containing Amies transport media (Becton, Dickinson and Company, USA) after opening the infection site. The pus/pus swab was labeled and transported to the laboratory immediately.

C. Nasal swab from dairy farmers

Swabs were collected from both nares of informed and consented dairy farm workers (Annex I-B and II-B) using BD culture swab and collection system containing Amies transport media (Becton, Dickinson and Company, USA). The sterile swab was inserted 2.5 cm (1 inch) from the edge of the nares, or until resistance is met at the level of the turbinates. It was then rotated 5 times against the anterior nasal mucosa and repeated with same swab in second naris. The nasal swabs were then returned back to its tube, labeled and transported to the Ayder Referal Hopsital Microbiology laboratory immediately.

D. Milk from dairy cows

Milk was collected from each lactating dairy cow (pooled milk sample of the 4 quarters for each cow) according to the procedures of the National Mastitis Council (http://www.nmconline.org/sampling.htm, accession date; May 26, 2014). Briefly, the udders of the cow was thoroughly cleaned with water and dried with a clean towel. Then teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to air dry. After discarding the first streams; three to four streams of milk (1-2 ml) from each udder quarter (4x3 = 12 streams in total from single cow) were collected in to sterile leak-proof plastic container and transported in an ice box to the Microbiology laboratory as soon as possible.

2.8. Laboratory Procedures

All the laboratory procedures performed for phenotypic and molecular characterizations of *S. aureus* isolated from humans and dairy cows' samples are outlined in the flowchart as shown Figure 2.2.

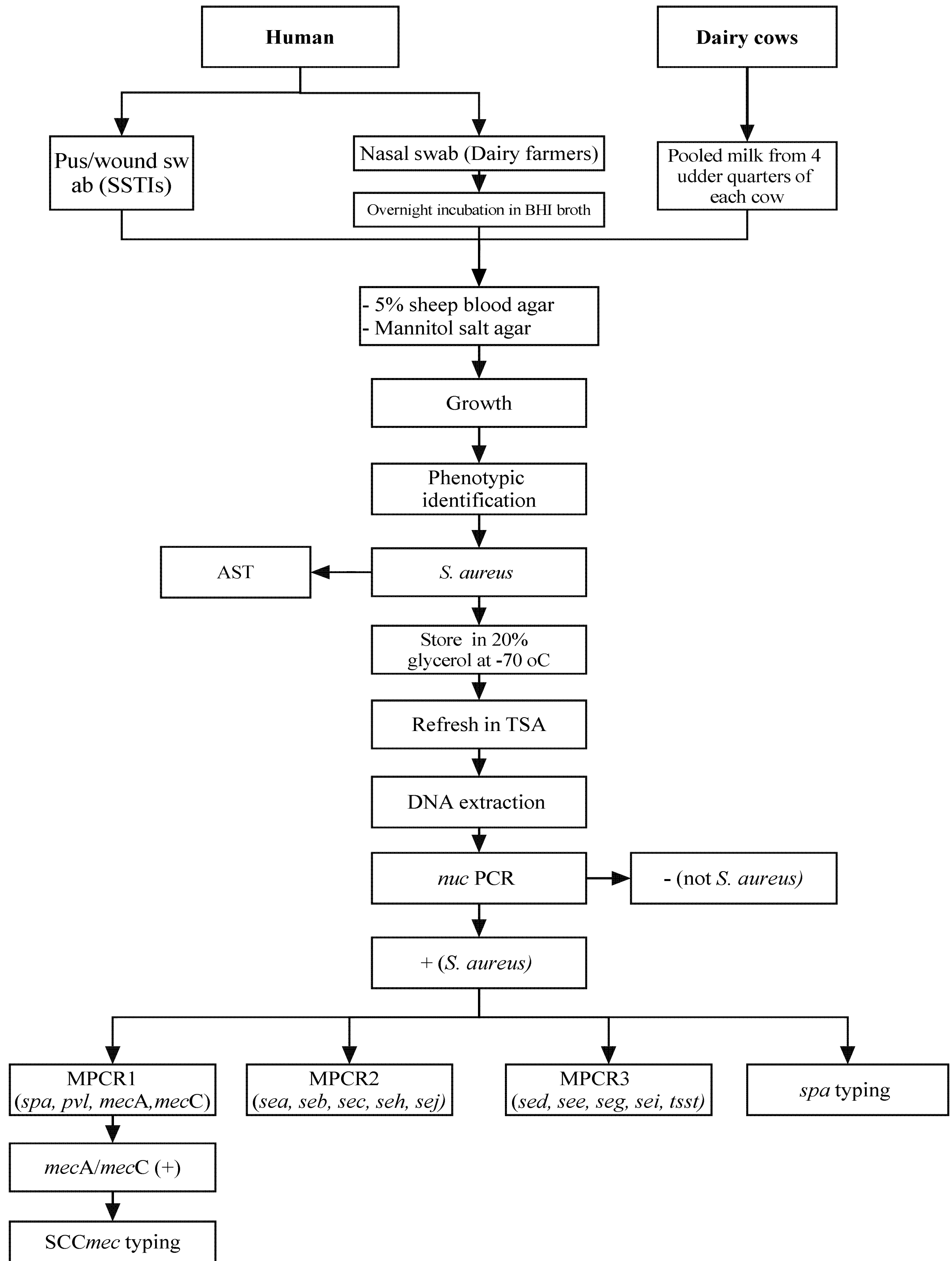


Figure 2.2: Laboratory flow chart for isolation, phenotypic and molecular characterization of *S. aureus*

2.8.1. Culture and Identification of *S. aureus*

All specimens were processed for culture and sensitivity testing at the Microbiology laboratory of Ayder referral hospital. Nasal swabs were initially incubated overnight in Brain Heart Infusion (BHI) broth (Oxoid, Ltd., England). Each specimen (wound swab, pus, 10 µl of milk and 100 µl of the BHI culture broth from nasal swabs were inoculated in to blood agar containing 5% sheep blood (Oxoid, Ltd., England) and Mannitol salt agar (Oxoid). Inoculated plates were incubated at 35-37°C in an aerobic atmosphere for 24 hours. After the incubation period, plates were inspected for bacterial growth; if no growth, incubation was extended to 48 hours. Colonies suspected as *S. aureus* were sub-cultured into Tryptic soy agar to get pure colonies and were identified as *S. aureus* based colony characteristics, Gram stain reaction (Gram positive cocci in clusters), hemolytic reaction, catalase test, coagulase test, DNase test and mannitol fermentation (Annex VI-A-C).

Phenotypically identified *S. aureus* isolates were stored in a storage media containing 20% glycerol/BHI broth at −70 ^{0}C until they were shipped to the Ohio State University, USA for molecular characterization. Shipping was done after gaining a material transfer agreement (MTA) signed by Addis Ababa University and the OSU (Annex-VII) and permission from the Ethiopian Biodiversity Institute.

2.8.2. Antimicrobial Susceptibility Testing

Following phenotypic identification, antimicrobial susceptibility testing (AST) was done for a panel of antimicrobial agents using disc diffusion technique and E-test according to the criteria of the Clinical and Laboratory Standards Institute [CLSI, 2014] (Annex VI-D). Briefly, a standardized suspension of each *S. aureus* isolate was prepared using normal saline and matched with the turbidity standard McFarland 0.5. The standardized suspension was streaked on to Muller-Hinton Agar (Oxoid) using sterile cotton tip applicator stick and allowed to dry. After that the antibiotic discs or E-test strips were placed on the medium and incubated at 35-37°C for 16-18 hours. For cefoxitin (30 µg) disc, the incubation time was extended to 24 hours. After the appropriate incubation time, the zones of inhibition were measured using caliper and interpreted as sensitive, intermediate and resistant.

For the disc diffusion method, the following antimicrobials and disc potencies were used: Penicillin (10 IU), Cefoxitin (30 µg), Erythromycin (15 µg), Tetracycline (30 µg), Clindamycin (2 µg), Trimethoprim-Sulphamethoxazole (1.25/23.75 µg), and Rifampin (5 µg).

Susceptibility testing for vancomycin and Daptomycin was done using the E-test method which determines the minimum inhibitory concentration (MIC). Inducible Clindamycin resistance was also performed for isolates that were erythromycin resistant but clindamycin susceptible using the double-disk diffusion test (D-test). Shortly, Clindamycin disk (2µg) and Erythromycin disk (15µg) were placed side by side approximately 15-26 mm apart. After overnight incubation, flattening of the zone of inhibition adjacent to the erythromycin disk was regarded as a positive D-test (inducible clindamycin resistance). Both the MIC determination and D-test were done and interpreted as per the CLSI criteria [CLSI, 2014].

The antimicrobial agents were selected based on the commonly prescribed antibiotics in the country and based on class representative antimicrobials stated in the CLSI Twenty-Third Informational Supplement on Performance Standards for Antimicrobial Susceptibility Testing [CLSI, 2014].

2.8.3. Molecular characterizations of *S. aureus* Isolates

All the molecular characterizations except *spa* typing were done at the Infectious Diseases Molecular Epidemiology Laboratory (IDMEL), Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, USA. *spa* typing of the entire *S. aureus* isolates was done at the Public Health Research Institute, International Center for Public Health, The State University of New Jersey, USA.

Molecular characterizations were done on the *S. aureus* isolates from humans and dairy cows to:

- Confirm phenotypically identified *S. aureus* isolates by *nuc* gene detection
- Determine the prevalence of MRSA by *mecA/mecC* detection and subsequently type them by SCC*mec* typing,

- Detect and identify pattern of *pvl*, enterotoxin genes and *tsst-1*
- Genotype and assess the clonal diversity and relatedness of the *S. aureus* isolates

I. DNA Extraction

Genomic DNA was extracted using DNeasy blood and tissue extraction kit for gram positive bacteria (Qiagen, Valencia, CA) following the manufacturer's instructions. Shortly, fresh colonies of *S. aureus* from an overnight grown culture on Tryptic soy agar (TSA) were harvested in to microcentrifuge containing 1.5 ml molecular grade water. The suspension was incubated at 37 ^{0}C for 30 minutes in lysis buffer (20 mM Tris·Cl, pH 8.0; 2 mM sodium EDTA; 1.2% Triton® X-100; lysozyme 20 mg/ml). After that, proteinase K and Buffer AL were added, mixed and incubated at 56°C for 30 minutes. Then, Ethanol (99.5%) was added and the mixture was pipetted in to DNeasy Mini spin column, centrifuged at 8000 rpm for 1 minute and the flow-through was discarded. The spin column was then placed in a new collection tube, 500 µl Buffer AW1 was added, centrifuged at 8000 rpm for 1 minute and flow-through was again discarded. The DNeasy Mini spin column was then placed in a new collection tube, 500 µl Buffer AW2 was added, centrifuged at 14,000 rpm for 3 minute and flow-through was discarded. Finally, the DNeasy Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, 100 µl molecular grade water was added, incubated at room temperature for 1 minute, and then centrifuged at 8000 rpm for 1 minute to elute. The elute was placed at -20 °C until used (Annex VI-E).

II. *nuc* gene detection

Phenotypically identified *S. aureus* isolates were confirmed by the detection of the thermonuclease coding gene, *nuc* according to [Brakstad *et al.*, 1992]. PCR amplification was performed on 25 µl reaction where 1 µl of each forward, reverse and template DNA and 22 µl of molecular grade water were added to the beads carefully and mixed well. The amplification included initial denaturation at 95°C for 4 minutes followed by 37 cycles (DNA denaturation at 95°C for 1 min, primer annealing at 55°C for 0.5 minute, DNA extension at 72°C for 1.5 minutes), and final extension at 72°C for 3.5 minutes. The PCR products were stored at 4°C until they were used. The sequences of the forward and reverse primers from 5' to 3' were GCGATTGATGGTGATACGGTT and

AGCCAAGCCTTGACGAACTAAAGC respectively. The expected amplified product size was 267 bp. A Negative control containing all the PCR mix except the template DNA and ATCC 29213 as a positive control were included in each PCR reaction [Annex V-A].

III. Multiplex PCR for the detection of *spa*, *pvl*, *mec*A, *mec*C genes

Detection of *spa*, *pvl*, *mec*A and *mec*C was performed as previously described protocol by [Stegger *et al.*, 2012] in 25 µl reaction using the illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA) [Annex V-B].

The following were added to the beads: 0.5 µl of each forward primer (2 µl total), 0.5 µl of each reverse primer (2 µl total), 2 µl of template DNA and 19 µl of molecular grade water. It was mixed by vortexing and amplified using PTC-100 thermocycler (MJ research inc., USA). The PCR conditions were: DNA denaturation at 94°C for 5 minutes followed by 30 cycles (DNA denaturation at 94°C for 30 seconds, primer annealing at 59°C for 1 minute, DNA extension at 72°C for 1 minute) and final elongation at 72°C for 10 minutes. The PCR products were stored at 4°C until they were used. Negative control containing all the PCR mix except the template DNA and positive controls were included in each PCR reaction. The control Strains and primers are listed in Table 2.1.

Table 2.1: Control strains and primer pairs for the multiplex PCR to detect *spa, pvl, mecA, mecC* genes of *S. aureus* isolated from humans and animals, Mekele, Ethiopia

<table>
<tr><th colspan="4">Control strains</th></tr>
<tr><th>Strain</th><th colspan="3">Target genes possessed</th></tr>
<tr><td>ATCC43300</td><td colspan="3">spa, mecA</td></tr>
<tr><td>MW2</td><td colspan="3">spa, pvl</td></tr>
<tr><td>LGA251</td><td colspan="3">spa, mecC</td></tr>
<tr><th colspan="4">Primers pairs</th></tr>
<tr><th>Genes</th><th>Nucleotide sequence (5'→ 3')</th><th>Amplified product size (bp)</th><th>Refe.</th></tr>
<tr><td>spa-1113F</td><td>TAAAGACGATCCTTCGGTGAGC</td><td rowspan="2">Variable (200-600 bp)</td><td rowspan="8">[Stegger et al., 2012]</td></tr>
<tr><td>spa-1514R</td><td>CAGCAGTAGTGCCGTTTGCTT</td></tr>
<tr><td>pvl-F</td><td>GCTGGACAAAACTTCTTGGAATAT</td><td rowspan="2">83</td></tr>
<tr><td>pvl-R</td><td>GATAGGACACCAATAAATTCTGGATTG</td></tr>
<tr><td>mecA P4</td><td>TCCAGATTACAACTTCACCAGG</td><td rowspan="2">162</td></tr>
<tr><td>mecA P7</td><td>CCACTTCATATCTTGTAACG</td></tr>
<tr><td>$mecA_{LGA251}$ MultiFP</td><td>GAAAAAAAGGCTTAGAACGCCTC</td><td rowspan="2">138</td></tr>
<tr><td>$mecA_{LGA251}$ MultiRP</td><td>GAAGATCTTTTCCGTTTTCAGC</td></tr>
</table>

IV. Multiplex PCR for the detection of *S. aureus* superantigen genes (*sea, seb, sec, sed, see, seg seh, sei, sej, tsst*)

Detection of *S. aureus* enterotoxin and toxic shock syndrome toxin genes was performed in 2 sets of multiplex PCRs as described by [Lovseth *et al.*, 2004]. Set 1 included *sea, seb, sec, seh, sej* and set 2 included *sed, see, seg, sei, tsst-1*). Each set of the multiplex PCR was done in 25 µl PCR reactions using the illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA) (Annex V-C, V-D).

Table 2.2: Control strains and primer pairs for the multiplex PCR to detect superantigen genes of *S. aureus* isolated from humans and animals, Mekele, Ethiopa

Control strains	
Reference strains	**Target genes possessed**
ATCC 14458	*seb*
ATCC 13565	*sea*
ATCC 25923	*seg, sei*
ATCC 23235	*sed, seg, sei, sej*
ATCC 19095	*sec, seh, seg, sei*
ATCC 27664	*see*
NRS 383	*seg, sei, tsst*

Primers pairs				
Primers		**Nucleotide sequence (5’→ 3’)**	**product size (bp)**	**Ref.**
MPCR set 1	*sea-F*	GCAGGGAACAGCTTTAGGC	521	[Lovseth *et al.*, 2004]
	sea-R	GTTCTGTAGAAGTATGAAACACG		
	seb-F	ACATGTAATTTTGATATTCGCACTG	667	
	seb-R	TGCAGGCATCATGTCATACCA		
	sec-F	CTTGTATGTATGGAGGAATAACAA	284	
	sec-R	TGCAGGCATCATATCATACCA		
	seh-F	CAACTGCTGATTTAGCTCAG	359	
	seh-R	GTCGAATGAGTAATCTCTAGG		
	sej-F	CATCAGAACTGTTGTTCCGCTAG	142	
	sej-R	CTGAATTTTACCATCAAAGGTAC		
MPCR set 2	*sed-F*	GTGGTGAAATAGATAGGACTGC	385	
	sed-R	ATATGAAGGTGCTCTGTGG		
	see-F	TACCAATTAACTTGTGGATAGAC	171	
	see-R	CTCTTTGCACCTTACCGC		
	seg-F	CGTCTCCACCTGTTGAAGG	328	
	seg-R	CCAAGTGATTGTCTATTGTCG		
	sei-F	CAACTCGAATTTTCAACAGGTACC	466	
	sei-R	CAGGCAGTCCATCTCCTG		
	tsst-F	GCTTGCGACAACTGCTACAG	559	
	tsst -R	TGGATCCGTCATTCATTGTTAT		

***MPCR: Multiplex PCR**

PCR mix for set 1 was prepared by adding 4.25 µl of forward primers (*sea* 0.75µl*, seb* 0.75µl*, sec* 1 µl*, seh* 1 µl*, sej* 0.75µl), 4.25 µl of reverse primers (*sea* 0.75µl*, seb* 0.75µl*, sec* 1 µl*, seh* 1 µl*, sej* 0.75µl = 4.25 µl) primers, 4 µl of template DNA and 12.5 µl molecular grade water to the beads. For set 2, the PCR mix was prepared by adding: 4µl of forward primers (*sed* 0.75µl*, see* 0.75µl*, seg* 0.75µl*, sei* 0.75µl*, tsst* 1 µl), 4µl of reverse primers (*sed* 0.75µl*, see* 0.75µl*, seg* 0.75µl*, sei* 0.75µl*, tsst* 1 µl), 4 µl of template DNA and 13 µl of molecular grade water to the beads. Amplification was performed using the PTC-100 thermocycler (MJ research inc., USA) by initial denaturation for 10 minutes at 95°C followed by 15 cycles (95°C for 1 minutes, 66°C for 45 seconds, 72° for 1 minutes), 20 cycles (95°C for 1 minutes, 63°C for 45 seconds, 72° for 1 minutes), and a final extension at 72°C for 10 minutes. The PCR products were stored at 4°C until they were used. Negative control containing all the PCR mix except the template DNA and positive controls were included in each PCR reaction. The nucleotide sequence of the forward and reverse primers and control strains are shown in Table 2.2.

V. SCC*mec* typing

SCC*mec* typing was performed according to previously described multiplex PCR by [Kondo *et al.*, 2007]. Three sets of multiplex PCRs were used for SCC*mec* type assignment and SCC*mec* IV sub-typing. The 1st MPCR was used to identify *ccr* gene types; the 2nd MPCR to identify *mec* classes, and 3rd MPCR to identify specific open reading frames in the J1 regions of type I and IV SCC*mec* elements. The SCC*mec* element type was defined by the combination of *ccr* type and *mec* class. The primers used for the SCC*mec* typing are shown in Table 2.3.

The 1st MPCR was used to identify the five types of *ccr* genes (*ccr1-ccr5*) (Annex V-E). Ten primers were used in this PCR where two were used to identify *mecA* (internal amplification control) and eight to identify the five *ccr* genes. PCR mixture was prepared by adding 0.5 µl of each the 10 primers (5 µl in total), 1 µl of template DNA and 19 µl of molecular grade water to the illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA).

Table 2.3: Control strains and primers for *S. aureus* SCC*mec* typing of MRSA isolated from humans and animals, Mekele, Northern Ethiopia

Control strains	
Reference strains	**Target gene/s possessed**
N315, JCSC3063	Type 2 *ccr*, class A *mec* = SCC*mec* type II
85/2082	Type 3 *ccr*, class A *mec* = SCC*mec* type III
MW2	Type 2 *ccr*, class B *mec* = SCC*mec* type IV
WIS	Type 5 *ccr*, class C2 *mec* = SCC*mec* type V
HDE288	Type 4 *ccr*, class B *mec* = SCC*mec* type VI
STO2984	Type IVa
STO2985	Type IVb
STO2986	Type IVc

Primers

	Primer list	Nucleotide sequence (5'→ 3')	Detected gene (Primer pair)	size (bp)	Ref
MPCR1	*mA1*	TGCTATCCACCCTCAAACAGG	*mecA (mA1- mA2)*	286	[Kondo *et al.*, 2007]
	mA2	AACGTTGTAACCACCCCAAGA			
	α1	AACCTATATCATCAATCAGTACGT	*ccrA1-ccrB (α1- βc)* = type 1 *ccr*	695	
	α 2	TAAAGGCATCAATGCACAAACACT	*ccrA2-ccrB (α2- βc)* = type 2 *ccr*	937	
	α 3	AGCTCAAAAGCAAGCAATAGAAT	*crA3-ccrB (α3- βc)* = type 3 *ccr*	1,791	
	βc	ATTGCCTTGATAATAGCCITCT			
	α4.2	GTATCAATGCACCAGAACTT	*ccrA4-ccrB4 (α4.2- β4.2)* = type 4 *ccr*	1,287	
	β4.2	TTGCGACTCTCTTGGCGTTT			
	γR	CCTTTATAGACTGGATTATTCAAAATAT	*ccrC (γR - γF)* = type 5 *ccr*	518	
	γF	CGTCTATTACAAGATGTTAAGGATAAT			
MPCR2	*mI6*	CATAACTTCCCATTCTGCAGATG	*mecA-mecI (mA7- mI6)* = class A	1,963	
	IS7	ATGCTTAATGATAGCATCCGAATG	*mecA*-IS*1272 (mA7-IS7)* = class B	2,827	
	IS2(iS-2)	TGAGGTTATTCAGATATTTCGATGT	*mecA*-IS*431 (mA7-IS2 [iS-2])* = class C	804	
	mA7	ATATACCAAACCCGACAACTACA			
MPCR3	*1a3*	TTTAGGAGGTAATCTCCTTGATG	E007 in type I.1 SCC*mec (1a3-la4)*	154	
	1a4	TTTTGCGTTTGCATCTCTACC			
	4al	TTTGAATGCCCTCCATGAATAAAAT	CQ02 in type IV.1 (IVa) SCC*mec* (*4al-4a3)*	458	
	4a3	AGAAAAGATAGAAGTTCGAAAGA			
	4b3	AACCAACAGTGGTTACAGCTT	M001 in type IV.2 (IVb) SCC*mec (4b3-4b4)*	726	
	4b4	CGGATTTTAGACTCATCACCAT			
	4c4	AGGAAATCGATGTCATTATAA	CR008 in type IV.3 (IVc) SCC*mec (4c4-4c5)*	259	
	4c5	ATCCATTTCTCAGGAGTTAG			
	4d3	AATTCACCCGTACCTGAGAA	D002 in type IV.4 (IVd) SCC*mec (4d3-4d4)*	1,242	
	d4	AGAATGTGGTTATAAGATAGCTA			

Amplification condition for the 1st MPCR included: initial denaturation at 94°C for 2 minutes; 30 cycles (denaturation at 94°C for 2 minutes, annealing at 57°C for 1 minutes, and extension at 72°C for 2 minutes); and a final elongation at 72°C for 2 minutes. Negative control containing all the PCR mix except the template DNA and positive controls were included in each PCR reaction as shown in Table 2.3.

The 2nd MPCR for the assignment of the 3 *mec* classes (class A to class C) contained four primers to identify the gene lineages of *mecA-mecI* (class A *mec*), *mecA*-IS*1272* (class B *mec*), and *mecA*IS*431* (class C *mec*) (Annex V-F). The PCR mixture was prepared by adding 0.5 µl of each the 4 primers (2 µl in total), 1 µl of template DNA and 22 µl of molecular grade water to the illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA). Amplification conditions included: initial denaturation at 94°C for 2 minutes; 30 cycles (denaturation at 94°C for 2 minutes, annealing at 60°C for 1 minutes, and extension at 72°C for 2 minutes); and a final elongation at 72°C for 2 minutes. Negative control containing all the PCR mix except the template DNA and positive controls were included in each PCR reaction as shown Table 2.3.

Table 2.4: **Interpretation of MPCR1 and MPCR2 for the assignment of SCC*mec* types**

MPCR1 (*ccr* type)	**MPCR2 (*mec*A class)**	**SCC*mec* type**	**Reference**
1	B	I	[Kondo *et al.*, 2007]
2	A	II	
3	A	III	
2	B	IV	
5	C2	V	
4	B	VI	

The 3rd MPCR which was used for SCC*mec* IV sub-typing, contained five primer pairs (Annex V-G): one pair for identifying specific ORF in the J1 region of type I SCC*mec* elements and four pairs for identifying specific ORFs in the J1 regions of four subtypes of type IV SCC*mec* elements. PCR mixture was prepared by adding 0.5 µl of each the 10 primers (5 µl in total), 1 µl of template DNA and 19 µl of molecular grade water to the illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA). Amplification conditions included: initial denaturation at 94°C for 2 minutes; 30 cycles (denaturation at 94°C for 2 minutes, annealing at 60°C for 1 minutes, and extension at 72°C

for 2 minutes); and a final elongation at 72°C for 2 minutes. Negative control containing all the PCR mix except the template DNA and positive controls were included in each PCR reaction as shown in Table 2.4.

VI. Agarose gel electrophoresis

All *nuc* gene and multiplex PCR products were analyzed using agarose gel electrophoresis. First, 2% Agarose gel was prepared by adding 2g of agarose powder to 100 ml 1x Tris-acetate-EDTA (TAE) in a microwavable flask. The solution was microwaved for 1-3 minutes until the agarose was completely dissolved. The agarose solution was cooled down to about 50°C and then Ethidium bromide (EtBr) was added to a final concentration of approximately 0.2-0.5 µg/ml (2-3 µl of lab stock solution per 100 ml gel). The agarose was poured into a gel tray with the well comb in place and allowed to completely solidify at room temperature for 20-30 minutes. Once solidified, the agarose gel was placed into the gel box (electrophoresis unit). The gel box was filled by 1x TAE until the gel was covered. Then a loading buffer was added to each of the PCR product samples. A molecular weight ladder was carefully loaded into the first lane of the gel, a negative control to the second lane and samples into the rest of the wells of the gel. The gel tank was closed with its lid and electrical leads were attached. The gel was run at 100 volts for 100 minutes. Then, the power was turned off, electrodes were disconnected from the power source, and the gel was carefully removed from the gel box, placed directly on a transilluminator for visualization of bands. For the *nuc* PCR, 1% agarose was used and the gel run was at 90 V for 60 minutes. The agarose gel electrophoresis protocol is shown in Annex VI-F.

VII. *spa* typing

spa-typing was performed for all confirmed *S. aureus* isolates as described previously by [Shopsin *et al.*, 1999] at the Public Health Research Institute, International Center for Public Health, Rutgers, The State University of New Jersey. Shortly, the polymorphic X region of *spa* gene was amplified by PCR and sequenced. Sequences were analyzed using Ridom Staph-Type software (Ridom GmbH), which automatically detects *spa* repeats and assigns a *spa*-type according to the Ridom Spa Server (http://spaserver.ridom.de/).

2.9. Quality Control

Pre-testing: Before the actual data collection, data collection sheets were pre-tested to ensure that whether the questions were understandably phrased; study participants understood the questions and instructions.

Specimen collection and transport: Sample collectors were trained on how to collect each sample (wound swab, pus, milk and nasal swab) and were instructed to follow standard operating procedure (SOP). Laboratory samples (wound swab, pus and nasal swabs) were collected using the BD culture swab and collection system (Becton, Dickinson and Company, USA) containing Amies transport media and milk samples were transported to the laboratory in an ice box within 6 hours of collection.

Laboratory processing of samples, culturing and sensitivity testing: Standard operating procedures (SOPs) and quality control measures were strictly followed. Culture Medias were sterilized based on the manufacturers' instructions. Sterility of culture media was cheeked by incubating 5% of the batch at 35-37°C overnight and observed for bacterial growth. If growth was observed the culture media were totally discarded. The quality of the culture media, gram stain and antimicrobial discs were checked using standard reference strains of *S. aureus* ATCC 29213 (MSSA), *S. aureus* ATCC 25923 and *Escherichia coli* (ATCC 25922).

Molecular techniques: Molecular tests were performed according to manufacturer instructions and standard operating procedures.

2.10. Data analysis

Data was entered into excel spreadsheet, cleaned and exported to SPSS software version 20 for analysis according to the study objectives. Descriptive analysis such as frequencies, means, proportions, standard deviations and graphs were calculated to describe some variables. Bivariate analyses such as x^2 test were used to describe the association between independent and dependent variables. *P-value<0.05* was considered as cut off point for significant association.

2.11. Ethical consideration

This PhD research project was approved and ethically cleared in the following order by:

- Department Ethical Review Committee (DERC) of the Microbiology, Immunology and Parasitology (DMIP), CHS, AAU, Ethiopia
- Institutional Review Board (AAU-IRB) of the Colleges of Health Sciences, Addis Ababa University, Ethiopia
- National Research Ethics Review Committee (NERC), Ministry of Science and Technology, Ethiopia
- Institutional Review Board of the Colleges of Health Sciences, Mekele University, Ethiopia.

In addition, permission was obtained from Tigray regional state health bureau, and the agriculture and development bureau of the region. Permission from dairy farm owners was obtained before collection of milk samples.

Written informed consent was obtained from each adult study participants. For children less than 18 years old, written informed consent was obtained from their parents/guardian. In addition, assent was also obtained for study participants between 12 and 18 years old. The aim of the study, its significance, confidentiality, participation right, procedure and associated risks were explained through an information sheet. Culture and sensitivity results were promptly reported to the attending physician for patient care. Moreover, findings of the present study will be communicated with responsible bodies for prevention and control measures and will be published on National and International Journals to disseminate information.

CHAPTER THREE: RESULTS

In this study a total of 811 non-duplicate specimens were obtained for *S. aureus* culture and identification; 355 wound/pus specimens from human skin and soft infections (SSTIs), 71 nasal swabs from dairy farmers and 385 milk samples from dairy cows.

3.1. Sociodemographic characteristics of the study participants

A. Human study participants with skin and soft tissue infections (SSTIs)

Among the 355 study participants with SSTIs, 217 (61%) were with clinically suspected community onset and 138 (39%) with hospital acquired infections. Majority of the study participants in this group were males constituting 219/355 (61.7%). The age of the study participants ranged from 3 months to 90 years and the mean age was 30.5 years. Majority of them were between the age of 16-30 years (146/355, 41.1%) followed by 31-45 years 74/355, 20.8%), above 45 years (70/355, 19.7%) and less than 15 years (65/355, 18.3%). About two third (222/355, 62.5%) came from urban area and obtained from 3 regional states; namely Tigray (329/355, 92.7%), Amhara (21/355, 5.9%) and Afar (5/355, 1.4%). Highest numbers of the study participants were from the Dermatology department of Ayder referral hospital (149/355, 42%) followed by adult Surgical ward (109/355, 30.7%), Orthopedic ward (73/355, 20.6%), Gynecology and Obstetrics ward 10/355 (2.8%), Pediatrics ward 9/355 (2.5%) and Medical ward 5/355 (1.4%) (Table 3.1).

I. Study participants with community onset SSTIs (human)

The community onset (CO)-SSTIs in this study included impetigo, folliculitis, furuncle, carbuncle, secondary bacterial infections of the skin and any infected open wound where onset of the infection was in the community. Of the 217 study participants suffering from community onset SSTIs, 138 (63.6%) were males. More than 70% of them were 30 years old or younger and around 30% were below 16 years old. Majority of them came from urban area (137/217, 63%) and were obtained from three hospital departments/wards; Dermatology OPD (149/217, 68.7%), orthopedics ward (63/217, 29%) and medical ward (5/217, 2.3%) as shown in Table 3.1.

II. Study participants with surgical site infections (SSI)

The hospital acquired (HA) SSTIs included 138 clinically suspected surgical site infections (SSIs) occurred in the hospital after surgery but before discharge. Of the 138 study participants suffering from SSIs, 81 (59%) were males. Almost all of the participants (137/138, 99.3%) were above 15 years old and 40/138 (29%) were older than 45 years. Most of the study participants were from urban area (85/138, 61.6%). They were obtained from the adult surgical ward (109/138, 79%), Gynecology and obstetrics ward (10/138, 7.2%), Orthopedics ward (10/138, 7.2%) and pediatric surgical ward (9/138, 6.5%) (Table 3.1).

Table 3.1: Sociodemographic characteristics of study participants with SSTIs in Ayder Referral Hospital, Mekele, Northern Ethiopia

Variables	SSTIs* (n=355)		Total,
	CO-SSTI (n=217)	SSI (n=138)	(n=355) (%)
Sex			
Male	138 (63.6)	81 (58.7)	219 (61.7)
Female	79 (36.4)	57 (41.3)	136 (38.3)
Age			
≤15 years	64 (29.5)	1 (0.7)	65 (18.3)
16-30 years	90 (41.5)	56 (40.6)	146 (41.1)
31-45	33 (15.2)	41 (29.7)	74 (20.8)
>45	30 (13.8)	40 (29.0)	70 (19.7)
Residence			
Rural	80 (36.9)	53 (38.6)	133 (37.5)
Urban	137 (63.1)	85 (61.6)	222 (62.5)
Region			
Tigray	205 (94.5)	124 (89.9)	329 (92.7)
Amhara	10 (4.6)	11 (8.0)	21 (5.9)
Afar	2 (0.9)	3 (2.2)	5 (1.4)
Hospital ward/Department			
Surgical A (Adult)	0 (0)	109 (79.0)	109 (30.7)
Dermatology	149 (68.7)	0(0)	149 (42)
GynyObs	0 (0)	10 (7.2)	10 (2.8)
Orthopedics	63 (29.0)	10 (7.2)	73 (20.6)
Surgical B (Pediatrics)	0 (0)	9 (6.5)	9 (2.5)
Medical	5 (2.3)	0 (0)	5 (1.4)

*SSTIs: Skin and Soft Tissue Infections

B. Lactating Dairy cows

A total of 385 dairy cows were included in the study. The dairy cows were selected from a total of 67 Small and large scale dairy farms located in 3 sub-cities of Mekele. About half of the cows were from Hawolti (200/385, 51.8%) and the rest were from Semen (101/385, 26.2%) and Hadnet (84/385, 21.8%). The age of the cows ranged from 3-14 years with the mean age of 5.9 years. Majority of the cows fall within the age group of 3-5 years (178/385, 46.2%) followed by 6-8 years (168/385, 43.6%) and ≥9 years (39/385, 10.1%). The parity of the cows ranged from 1-11 where two third (254/385, 66%) gave birth to 1-3 calves. More than half of the cows (203/385, 52.7%) had less than three months of lactation stage. Twelve cows (3.1%) had taken antibiotics within the past one year. Milking process was conducted manually in all lactating cows (Table 3.2).

Table 3.2: Characteristics of Dairy cows from 67 small and large scale dairy farms in Mekele, Northern Ethiopia

Variables	Sub-city and number of lactating cows			Total, n/385 (%)
	Hadnet (n=84) (%)	Hawolti (n=200) (%)	Semen (n=101) (%)	
Age group in years				
3-5 years	39 (46.4)	94 (47.0)	45 (44.6)	178 (46.2)
6-8 years	34 (40.5)	87 (43.5)	47 (46.5)	168 (43.6)
≥9 years	11 (13.1)	19 (9.5)	9 (8.9)	39 (10.1)
Parity				
1-3	52 (61.9)	135 (67.5)	67 (66.3)	254 (66.0)
4-6	28 (33.3)	53 (26.5)	32 (31.7)	113 (29.4)
> 6	4 (4.8)	12 (6.0)	2 (2.0)	18 (4.7)
Lactation				
<3 month	59 (70.2)	90 (45.0)	54 (53.5)	203 (52.7)
3-6 month	25 (29.8)	63 (31.5)	39 (38.6)	127 (33.0)
>6 month	0 (0)	47 (23.5)	8 (7.9)	55 (14.3)
Antibiotics intake within the last 1 year				
Yes	0 (0)	11 (5.5)	1 (1.0)	12 (3.1)
No	84 (100)	189 (94.5)	100 (99.0)	373 (96.9)

C. Dairy farmers

Data was collected from 71 dairy farmers working in the 67 dairy farms. More than three fourth (55/71, 77.5%) of the workers were males and majority of them were attendants (62/71, 87.3%). Their age ranged from 17 to 63 years with the mean age of 29 years. Their work experience in the current farm ranged from 1 month to 40 years where more than 70% had less than or equal to 5 years of experience. None of them had taken any antibiotics in the past two weeks (Table 3.3).

Table 3.3: Sociodemographic characteristics of Dairy farm workers from 67 small and large scale dairy farms in Mekele, Northern Ethiopia

Variables	Frequency (n)	Percent (n/71*100)
Sex		
Male	55	77.5
Female	16	22.5
Age in years, n=71		
17-27	45	63.4
28-38	10	14.1
39-49	7	9.9
50-63	9	12.7
Farm Duty, n=71		
Attendant	62	87.3
Owner	9	12.7
Work experience in the current dairy farm, n=71		
≤5 years	50	70.4
6-10 years	14	19.7
11-15 years	1	1.4
16-20 years	4	5.6
≥21 years	2	2.8

3.2. Isolation rate of Staphylococcus aureus

S. aureus was isolated from different specimens obtained from 193 of the 811 study participants with an overall prevalence of 23.8%. The prevalence of *S. aureus* varies with study group where a higher isolation rate was documented from study participants with skin

and soft tissue infections (123/355, 34.6%) followed by dairy farmers (22/71, 31%) and dairy cows (48/385, 12.5%). Moreover, *S. aureus* was isolated from 88/217 (40.5%) of study participants with community-onset SSTIs as compared to 35/138 (25.4%) from participants with hospital-onset SSTIs (Table 3.4).

Table 3.4: Frequency of *S. aureus* isolation from the different study groups in Mekele, Northern Ethiopia

Study groups		Total samples processed	Number of *S. aureus* isolated	Total (%)
Participants with SSTI	CO-SSTI	217	88	88/217 (40.5)
	SSI	138	35	35/138 (25.4)
	Sub-total	**355**	**123**	**123/355 (34.6)**
Dairy farmers (DF)		71	22	22/71 (31.0)
Dairy cattle (DC)		385	48	48/385 (12.5)
Total (SSTI+DF+DC)		**811**	**193**	**193/811 (23.8)**

Key: CO-SSTI= Community onset skin and soft tissue infection**, SSI =** surgical site infection

3.3. Antimicrobial susceptibility data

All the *S. aureus* isolates (n=193) were tested for a panel of nine antimicrobial agents. Overall, higher frequency of resistance was recorded for penicillin (179/193, 92.7%) followed by tetracycline (74/193, 38.3%), trimethoprim-Sulphamethoxazole (61/193, 31.6%), trythromycin (36/193, 18.7%), clindamycin (10/193, 5.2%) and cefoxitin (4/193, 2.1%). No resistance was observed against vancomycin, daptomycin and rifampin. Among the four cefoxitin resistant (Methicillin resistant *S. aureus*, MRSA) isolates, three were from human study participants with Skin and soft tissue infections and one from nasal of dairy farmer. No MRSA was isolated from milk of the dairy cows (Table 3.5). Resistance to erythromycin (31/193, 25%, *p= 0.002*) and trimethoprim-Sulfamethoxazole (50/193, 40.7%, *p=0.001*) was found significantly higher among human clinical isolates than dairy farm isolates.

Overall, 183/193 (94.8%) of the isolates were resistant to at least one of the tested nine antimicrobial agents. Seventy six (39.4%) isolates were resistant to only one antimicrobial

agent and 107/193 (55.4%) were resistant to two or more antimicrobial classes (Figure 3.1 and Table 3.6).

Table 3.5: Antimicrobial susceptibility profile of 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia

Antibiotic & Concentration	Interpretation	SSTIs*			Dairy farmers	Dairy cows	Total
		CO-SSTI	HA-SSTI	Sub-total			
Penicillin (10µg)	S*	7(8.0)	1(2.9)	8(6.5)	2(9.1)	4(8.3)	14(7.3)
	R*	81(92.0)	34(97.1)	115(93.5)	20(90.9)	44(91.7)	179(92.7)
Cefoxitin (30µg)	S	86(97.7)	34(97.1)	120(97.6)	21(95.5)	48(100)	189(97.9)
	R	2(2.3)	1(2.9)	3(2.4)	1(4.5)	0(0)	4(2.1)
Erythromycin (15µg)	S	58(65.9)	20(57.1)	78(63.4)	18(81.8)	44(91.7)	140(72.5)
	I*	12(13.6)	2(5.7)	14(11.4)	0(0)	3(6.2)	17(8.8)
	R	18(20.5)	13(37.1)	31(25.2)	4(18.2)	1(2.1)	36(18.7)
Clindamycin (2µg)	S	81(92.0)	30(85.7)	111(90.2)	22(100)	47(97.9)	180(93.3)
	I	2(1.7)	1(2.9)	3(2.4)	0(0)	0(0)	3(1.6)
	R	5(5.7)	4(7.3)	9(7.3)	0(0)	1(2.1)	10(5.2)
Tetracycline (30µg)	S	50(56.8)	23(65.7)	73(59.3)	15(68.2)	28(58.3)	116(60.1)
	I	0(0)	0(0)	0(0)	0(0)	3(6.2)	3(1.6)
	R	38(43.2)	12(34.3)	50(40.7)	7(31.8)	17(35.4)	74(38.3)
Trimethoprim-Sulfamethoxazole (1.25/23.75 µg)	S	46(52.3)	19(54.3)	65(52.8)	16(72.7)	41(85.4)	122(63.2)
	I	7(8.0)	1(2.9)	8(6.5)	0(0)	2(4.2)	10(5.2)
	R	35(39.8)	15(42.9)	50(40.7)	6(27.3)	5(10.4)	61(31.6)
Rifampin (5µg)	S	88 (100)	35 (100)	123(100)	22(100)	48(100)	193(100)
	R	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Vancomycin E-test	S	88 (100)	35 (100)	123(100)	22(100)	48(100)	193(100)
	R	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Daptomycin E-test	S	88 (100)	35 (100)	123(100)	22(100)	48(100)	193(100)

Key: CO-SSTI: Community onset Skin and Soft Tissue Infection; HA-SSTI: Hospital Acquired Skin and Soft Tissue Infection, S: Sensitive; I: Intermediate; R: Resistant

Multidrug drug resistance (resistance to three or more antimicrobial classes) was documented on 50/193(25.9%) of the isolates. The frequency and percentage of resistance to two, three, four, five and six antimicrobial agents was 57/193(29.5%), 32/193(16.6%),

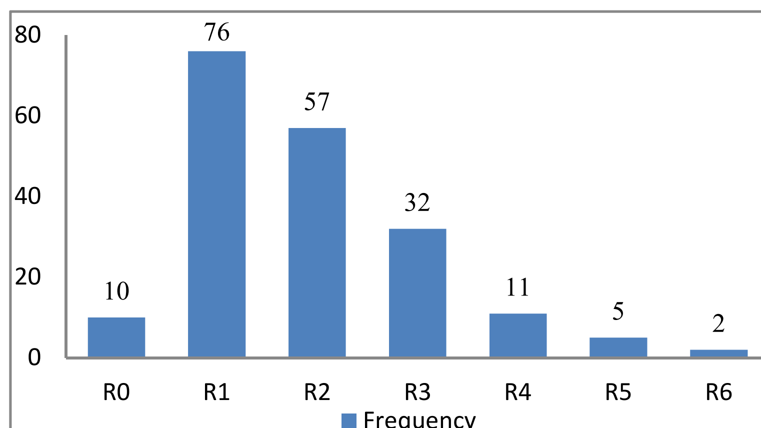
11/193(5.7%), 5/193(2.6%) and 2/193(1.0%), respectively. The methicillin resistant *S. aureus* (MRSA) isolates were resistant to at least four classes of antimicrobials where two of them were resistant to six antimicrobials (Figure 3.1 and Table 3.6).

Table 3.6: Antibiogram profile of 193 *S. aureus* isolates from humans and dairy cows in Mekele, Northern Ethiopia

Resistance to Antimicrobials	Number of strains	Percent (%)
R0	10	5.2
P	72	37.3
T	2	1.0
P+E	9	4.7
P+SXT	21	10.9
P+T	29	15.0
P+E+CL	1	0.5
P+E+SXT	4	2.1
P+E+T	6	3.1
P+T+SXT	23	11.9
P+CF+E+T	1	0.5
P+E+CL+SXT	2	1.0
P+E+CL+T	2	1.0
P+E+T+SXT	5	2.6
P+CF+E+T+SXT	1	0.5
P+E+CL+T+SXT	3	1.6
P+CF+E+CL+T+SXT	2	1.0
Total	**193**	**100**

Key: R0: susceptible to all, P: penicillin, CF: cefoxitin, CL: clindamycin, E: Erythromycin, SXT: Trimethoprim-Sulphamethoxazole, T: tetracycline

Figure 3.1: Resistance pattern out of the nine antimicrobials tested for the 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia.



Key:

R0: susceptible to all drugs

R1: Resistant to only one drug

R2: Resistant to two drugs

R3: Resistant to three drugs

R4: Resistant to four drugs

R5: Resistant to five drugs

R6: Resistant to six drugs

3.4. Toxin genes profile of *S. aureus* isolates

A. Overall toxin genes detected

All the *S. aureus* isolates (n=193) were tested for the presence of 11 major toxin genes; panton-valentine leukocidin toxin (*pvl*), toxic shock syndrome toxin-1 (*tsst-1*), and nine major staphylococcal enterotoxins (*sea, seb, sec, sed, see, seg, seh, sei, sej*). Among the 193 *S. aureus* isolates, 129 (66.8%) possessed at least 1 of the 11 targeted toxin genes. Staphylococcal enterotoxin genes (*se*) were detected in 108/193 (56%) isolates and classical enterotoxin genes in 74/193 (38.3%) of the isolates as shown in Table 3.7.

Table 3.7: Percentage of toxin genes positivity of the 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia

Toxin genes		Sources of isolate			Total (n=193)	*p-value*
		Human SSTIs (n=123)	Nares of dairy farmers (n=22)	Milk of dairy cows (n=48)		
Possession of at least one of 11 toxin genes	Yes	100 (81.3%)	14 (63.6%)	15 (31.2%)	129 (66.8%)	*<0.001*
	No	23 (18.7%)	8 (36.4%)	33 (68.8%)	64 (33.2%)	
Possession of at least one of the nine enterotoxin genes	Yes	81(65.9%)	12 (54.5%)	15 (31.2%)	108 (56%)	*<0.001*
	No	42 (34.1%)	10 (45.5%)	33 (68.8%)	85 (44%)	
Possession of at least one of the five classical enterotoxin genes	Yes	60 (48.8%)	8 (36.4%)	6 (12.5%)	74 (38.3)	*<0.001*
	No	63(51.2%)	14 (63.6%)	42 (87.5%)	119 (61.7%)	

As shown in Table 3.8, the most frequently detected toxin gene was *pvl* (71/193, 36.8%), followed by *seg* (56/193, 29%), *sei* (56/193, 29%), *seb* (37/193, 19.2%), *sea* (31/193, 16%) and *sec* (29/193, 15%). *seh* (8/48, 16.7%) followed by *sea* (5/48, 10.4%) and *sej* (5/48, 10.4%) were the most frequently detected toxin genes from milk of dairy cows.

Table 3.8: Toxin genes profile of the 193 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia

Toxin genes		Sources of isolates			Total (n=193)	*p-value*
		Human SSTIs (n=123)	Nares of dairy farmers (n=22)	Milk of dairy cows (n=48)		
pvl	Yes	66 (53.7%)	3 (13.6%)	2 (4.2%)	71 (36.8%)	*<0.001*
	No	57 (46.3%)	19 (86.4%)	46 (95.8%)	122 (63.2%)	
tsst-1	Yes	15 (12%)	4 (18.2%)	0 (0%)	19 (9.8%)	*0.021*
	No	108 (88%)	18 (81.8%)	48 (100%)	174 (90.2%)	
sea	Yes	24 (19.5%)	2 (9.1%)	5 (10.4%)	31 (16%)	*0.222*
	No	99 (80.5%)	20 (90.9%)	43 (89.6%)	162 (84%)	
seb	Yes	28 (22.8%)	5 (22.7%)	4 (8.3%)	37 (19.2%)	*0.089*
	No	95 (77.2%)	17 (77.3%)	44 (91.7%)	156 (80.8%)	
sec	Yes	22 (18%)	3 (13.6%)	4 (8.3%)	29 (15%)	*0.286*
	No	101 (82%)	19 (86.4%)	44 (91.7%)	164 (85%)	
sed	Yes	11 (9%)	1 (4.5%)	4 (8.3%)	16 (8.3%)	*0.789*
	No	112 (91%)	21 (95.5%)	44 (91.7%)	177 (91.7%)	
see	Yes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-----
	No	123 (100%)	22 (100%)	48 (100%)	193 (100%)	
seg	Yes	46 (37.4)	6 (27.3%)	4 (8.3%)	56 (29%)	*0.001*
	No	77 (62.6%)	16 (72.7%)	44 (91.7%)	137 (71%)	
seh	Yes	19 (15.4%)	1 (4.5%)	8 (16.7%)	28 (14.5%)	*0.363*
	No	104 (84.6%)	21 (95.5%)	40 (83.3%)	165 (85.5%)	
sei	Yes	46 (37.4%)	6 (27.3%)	4 (8.3%)	56 (29%)	*0.001*
	No	77 (62.6%)	16 (72.7%)	44 (91.7%)	137 (71%)	
sej	Yes	14 (11.4%)	2 (9.1%)	5 (10.4%)	21 (10.9%)	*0.944*
	No	109 (88.6%)	20 (90.9%)	43 (89.6%)	172 (89.1%)	

SSTIs: Skin and Soft Tissue Infections

B. Combination of toxin genes detected

Out of the 193 *S. aureus* isolates, 43 (22.3%) carried only one of the 11 targeted toxin genes and 86/193 (44.6%) possessed two or more (2-8). Among the *S. aureus* isolates carrying two or more toxin genes, 28/193 (14.5%) had two, 19/193 (9.8%) had three, 19/193 (9.8%) had four, 5/193 (2.6%) had five, 1/193 (0.5%) had six, 11/193 (5.7%) had seven and 3/193 (1.5%) had eight toxin genes (Figure 3.2).

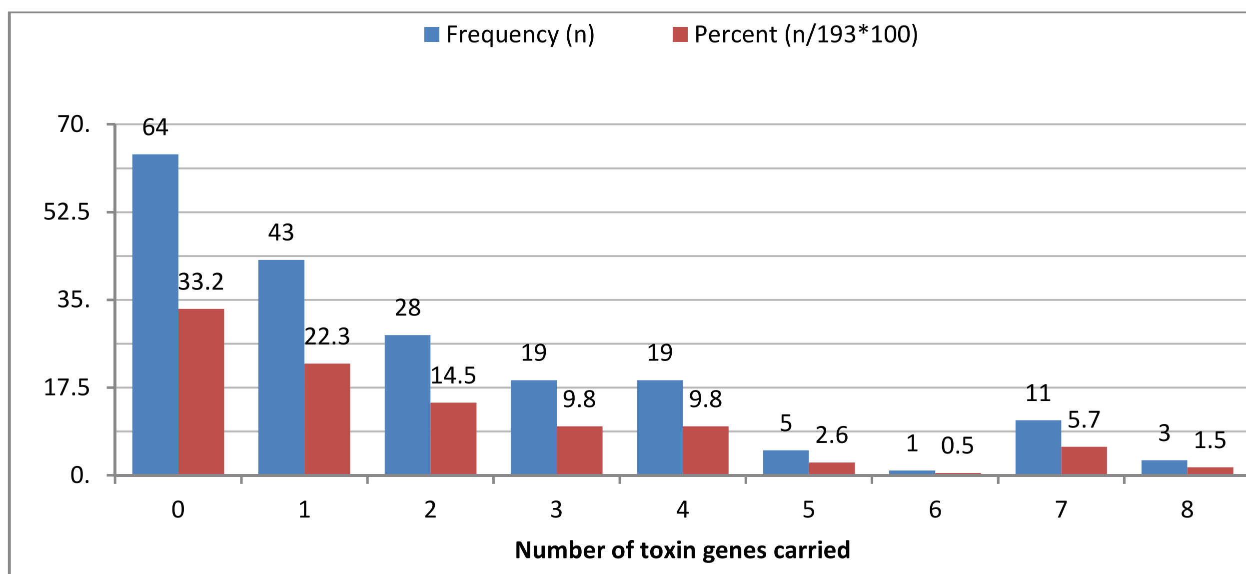
Table 3.9: Combinations of toxin genes carried by *S. aureus* isolates from different sources, Mekele Northern Ethiopia

No of toxin genes carried	Toxin gene type/Combination	Source of isolates			Total	Percent (T/193*100)
		Milk	Nasal	SSTI		
0	None	33	8	23	**64**	**33.2**
1	*pvl*	0	2	19	**21**	**10.9**
	sea	0	1	3	**4**	**2.1**
	seb	0	2	1	**3**	**1.6**
	seh	8	1	4	**13**	**6.7**
	sej	1	1	0	**2**	**1.0**
2	*pvl+sea*	2	0	10	**12**	**6.2**
	pvl+seb	0	0	1	**1**	**0.5**
	pvl+sec	0	0	1	**1**	**0.5**
	pvl+seh	0	0	2	**2**	**1.0**
	pvl+sej	0	0	3	**3**	**1.6**
	sea+seh	0	0	2	**2**	**1.0**
	sea+tsst-1	0	1	0	**1**	**0.5**
	seg+sei	0	0	6	**6**	**3.1**
3	*pvl+sea+seh*	0	0	5	**5**	**2.6**
	pvl+sec+seh	0	0	3	**3**	**1.6**
	pvl+seg+sei	0	0	4	**4**	**2.1**
	seb+seg+sei	0	0	2	**2**	**1.0**
	sec+seg+sei	0	1	1	**2**	**1.0**
	seg+sei+tsst-1	0	2	1	**3**	**1.6**
4	*pvl+seb+seg+sei*	0	1	10	**11**	**5.7**
	pvl+sec+seg+sei	0	0	1	**1**	**0.5**
	sea+sec+seg+sei	0	0	1	**1**	**0.5**
	seb+sec+seg+sei	0	1	0	**1**	**0.5**
	seb+seg+sei+tsst-1	0	0	2	**2**	**1.0**
	sec+seg+seh+sei	0	0	1	**1**	**0.5**
	sed+seg+sei+sej	0	0	1	**1**	**0.5**
	seg+seh+sei+tsst-1	0	0	1	**1**	**0.5**
5	*pvl+sea+sec+seg+sei*	0	0	1	**1**	**0.5**
	pvl+sea+seg+sei+tsst-1	0	0	1	**1**	**0.5**
	pvl+seb+sec+seg+sei	0	0	2	**2**	**1.0**
	pvl+sec+seg+seh+sei	0	0	1	**1**	**0.5**
6	*seb+sec+sed+seg+sei+sej*	1	0	0	**1**	**0.5**
7	*sea+seb+sec+sed+seg+sei+sej*	3	0	0	**3**	**1.6**
	seb+sec+sed+seg+sei+sej+tsst-1	0	1	7	**8**	**4.1**
8	*pvl+seb+sec+sed+seg+sei+sej+tsst-1*	0	0	2	2	1.0
	sea+seb+sec+sed+seg+sei+sej+tsst-1	0	0	1	1	0.5

SSTI: skin and soft tissue infection

Among the toxin genes detected *pvl, sea, seb, seh* and *sej* were found either alone or in combination with the other toxin genes. However, *sec, sed, seg, sei and tsst-1* were found only in combination with other genes. The number and combination of toxin genes possessed by the 129 toxin positive *S. aureus* isolates are shown in Table 3.9.

Figure 3.2: Toxin gene carriage out of the 11 target genes by the 193 *S. aureus* isolates from Mekele, Northern Ethiopia



C. Individual toxin gene profile of *S. aureus*

I. Panton-valentine leukocidin toxin gene (*pvl*)

In this study, *Pvl* was the most frequently detected toxin gene carried by 71 of the 193 *S. aureus* isolates (36.8%) (Table 3.8). However, significant difference was observed on *pvl* carriage among the *S. aureus* isolates based on their source. Briefly, *pvl* carriage was significantly higher ($p<0.001$) on *S. aureus* clinical isolates from skin and soft tissue infections (66/123, 53.7%) compared to isolates from nares of dairy farmers (3/22, 13.6%) and cows' milk (2/48, 4.2%) as shown in Table 3.8. *pvl* was found either alone or in combination with each of the other toxin genes (Table 3.9).

II. Toxic shock syndrome toxin-1 gene *(tsst-1)*

The overall prevalence of *tsst-1* among the 193 *S. aureus* isolates was 9.8% (Table 3.8). However, *tsst-1* was detected only on human *S. aureus* isolates but not from cows' milk.

Among the *tsst-1* positive isolates, 15/123 (12%) were form skin and soft tissue infections and 4/22 (18.2%) were from nares of farm workers (Table 3.7). *S. aureus* isolates that possess *tsst-1* also possessed at least one of the other toxin genes (Table 3.9).

III. Staphylococcal enterotoxin genes *(se)*

Out of 193 *S. aureus* isolates, 108 (56%) were found to be positive for at least one of the nine enterotoxin (*se*) genes (Table 3.7). Possession of at least one *se* was higher in ($p<0.001$) isolates of human skin and soft tissue infections (81/123, 66%) and nares of dairy farmers (12/22, 54.5%) than isolates from milk of dairy cows (15/48, 31.2%). Among the 9 enterotoxin genes, *seg* and *sei* (each detected on 56/193, 29% of isolates) were the dominant toxin genes followed by *seb* (37/193, 19.2%), *sea* (31/193, 16%), *sec* (29/193, 15%), *seh* (28/193, 14.5%), *sej* (21/193, 10.9%) and *sed* (16/193, 8.3%). No *S. aureus* isolate was found to be positive for *see* in this study. Higher proportion of *S. aureus* isolates from human skin and soft tissue infections possessed at least one enterotoxin gene (81/123, 65.9%) compared to isolates from nares of farm workers (12/22, 54.5%) and milk of dairy cows (15/48, 31.2%) as indicated in Table 3.7.

Among the enterotoxin genes *seg* and *sei* were found significantly higher in *S. aureus* isolates from skin and soft tissue infections and nares of farm workers compared to dairy cows' milk isolates ($p=001$). However, *seh* was the leading toxin gene detected on milk isolates compared to human isolates although the difference was not statistically significant ($p=0.363$) [Table 3.8].

Out of the enterotoxin gene positive isolates, 44/193 (22.8%) possessed only one of the 9 targeted enterotoxin genes and 64/193 (31.2%) possessed two or more (2-7 genes). The *sea*, *seb*, *seh* and *sej* existed either alone or in combination with other enterotoxin genes. However, *seg* and *sei* always existed together and *sed* was found to be always in combination with *sej* (Table 3.10).

Table 3.10: Combination of staphylococcal enterotoxin (*se*) genes of the 193 *S. aureus* isolates from Mekele, Northern Ethiopia

No of *se* carried out of 9	Types of *se* gene possessed	Source of isolate			Total	Percent
		Milk	Nasal	SSTI	(T)	(T/193*100)
0	none	33	10	42	**85**	**44.0**
1	*sea*	2	2	13	**17**	**8.8**
	seb	0	2	2	**4**	**2.1**
	sec	0	0	1	**1**	**0.5**
	seh	8	1	6	**15**	**7.8**
	sej	1	1	3	**5**	**2.6**
2	*sea+seh*	0	0	7	**7**	**3.6**
	sec+seh	0	0	3	**3**	**1.6**
	seg+sei	0	2	11	**13**	**6.7**
3	*sea+seg+sei*	0	0	1	**1**	**0.5**
	seb+seg+sei	0	1	14	**15**	**7.8**
	sec+seg+sei	0	1	2	**3**	**1.6**
	seg+seh+sei	0	0	1	**1**	**0.5**
4	*sea+sec+seg+sei*	0	0	2	**2**	**1.0**
	seb+sec+seg+sei	0	1	2	**3**	**1.6**
	sec+seg+seh+sei	0	0	2	**2**	**1.0**
	sed+seg+sei+sej	0	0	1	**1**	**0.5**
6	*seb+sec+sed+seg+sei+sej*	1	1	9	**11**	**5.7**
7	*sea+seb+sec+sed+seg+sei+sej*	3	0	1	**4**	**2.1**

SSTIs: Skin and Soft Tissue Infections

IV. Staphylococcal classical enterotoxin (*se*) genes

Out of 193 *S. aureus* isolates, 74 (38.3%) were found positive for at least one of the five classical enterotoxin genes (*sea, seb, sec, sed* and *see*). Among these genes, *seb* was the most dominantly detected gene (37/193, 19.2%) followed by *sea* (31/193, 16%), *sec* (29/193, 15%), and *sed* (16/193, 8.3%) (Table 3.8). It was observed that the classical enterotoxin genes existed independent of one another (Table 3.11).

The findings of the present study indicated that the overall classical enterotoxin gene positivity was higher on human SSTI isolates (60/123, 48.8%) and nares of farm workers (8/22, 36.4%) compared to dairy cows' milk isolates (6/48, 12.5%) [Table 3.7]. However, there was no statisticaly significant difference on the possession of the individual classical enterotoxin genes possession among the different study groups. However, *seb* possession was higher on human isolates (33/145, 22.8%) compared to cow isolates (4/48, 8.3%) although not statistically significant ($p = 0.062$) [Table 3.7].

Table 3.11: Combination of classical enterotoxin genes carried by the 193 *S. aureus* isolates from Mekele, Northern Ethiopia

Number of classical *se* possessed	Types of classical *se* possessed	Source of isolates			Total	Percent
		Milk	Nasal	SSTI		
0	None	42	14	63	**119**	**61.7**
1	*sea*	2	2	21	**25**	**13.0**
	seb	0	3	16	**19**	**9.8**
	sec	0	1	8	**9**	**4.7**
	sed	0	0	1	**1**	**0.5**
2	*sea+sec*	0	0	2	**2**	**1.0**
	seb+sec	0	1	2	**3**	**1.6**
3	*seb+sec+sed*	1	1	9	**11**	**5.7**
4	*sea+seb+sec+sed*	3	0	1	**4**	**2.1**

Among the 193 isolates, 54 (28%) possessed only one type of classical enterotoxin gene and the remaining 20 isolates (10.4%) possessed two or more. Among those possessing two or more, 5/193 (2.6%) possessed two, 11/193 (5.7%) possessed three and 4/193 (2.1%) possessed four. Three (75%) of the four *S. aureus* isolates that carried four of the classical enterotoxin genes were isolated from milk of dairy cows (Table 3.11).

3.5. *mec* genes carriage and *SCCmec* typing

Out of the 193 *S. aureus* isolates, *mecA* was detected in four (2.1%) isolates but none of the isolates carried *mecC*. All the *mecA* positive isolates were from human; two of them were isolated from community onset SSTIs, one from surgical site infection and the other one

from nare of dairy farm worker. Two of them were *spa* type t037 and the rest were t064, t1855. Regarding their clonal complex, two were CC239, one CC8 and the other one CC88. *SCCmec* typing showed that two of them were *SCCmec* type III and the rest two *SCCmec* type Iva.

3.6. *spa* typing result

Out of 193 *S. aureus* isolates, 190 (98.4%) were successfully typed using the Ridom *spa* typing method (121 from humans, 22 from dairy farmers and 47 from dairy cows) as shown in Table 3.12. The *spa* typing resulted in 61 different types. *S. aureus* isolated from human were found to be highly diverse (50 different *spa* types from SSTI isolates and 19 different *spa* types of nasal isolates) compared to dairy cow isolates (only 7 different *spa* types). Generally, *spa* type t042 (31/190, 16.3%) was the most frequently detected followed by t355 (24/190, 12.6%), t306 (14/190, 7.4%), t084 (10/190, 5.3%), t085 (10/190, 5.3%), t2856 (10/190, 5.3%), t127 (6/190, 3.2%), and t314 (5/190, 2.6%). Out of the 61 different *spa* types identified, five were reported for the first time in this study; i.e. *spa* type t17828, t17829, t17830, t17831, t17832 and all of them were human isolates.

Of the 50 different *spa* types of 121 human clinical *S. aureus* isolates, t355 was found to be the most dominant (23/121, 19%) followed by t306 (10/121, 8.3%), t084 (8/121, 6.6%), t085 (8/121, 6.6%), t127 (6/121, 5%) and t314 (5/121, 4.1%). Two of the five newly reported *spa* types, t17829 and t17830, were obtained from human study participants with SSTIs (Table 3.12).

The *spa* typing data showed that *S. aureus* isolates of nasal origin were the most genetically diverse where 19 different *spa* types were documented from 22 isolates compared to the SSTIs isolates (50 *spa* types from 121 isolates) and milk isolates (7 *spa* types from 47 isolates). This means all nasal *S. aureus* isolates except three (t084, t223 and t701) had unique *spa* type.

Table 3.12: Pattern of *spa* types of the 190 *S. aureus* isolates from human and dairy cows in Mekele, Northern Ethiopia

***Spa* type**	**Source of isolates**			**Total**	**%**
	Milk	**Nasal**	**SSTI**		
t042	29	1	1	31	16.3
t355	0	1	23	24	12.6
t306	3	1	10	14	7.4
t084	0	2	8	10	5.3
t085	2	0	8	10	5.3
t2856	9	1	0	10	5.3
t127	0	0	6	6	3.2
t314	0	0	5	5	2.6
t002	0	1	3	4	2.1
t6218	0	0	4	4	2.1
t186	0	1	2	3	1.6
t223	0	2	1	3	1.6
t261	0	0	3	3	1.6
t5338	1	1	1	3	1.6
t701	0	2	1	3	1.6
t941	0	1	2	3	1.6
t037	0	0	2	2	1.1
t062	0	0	2	2	1.1
t1977	0	0	2	2	1.1
t272	0	0	2	2	1.1
t279	0	0	2	2	1.1
t325	0	0	2	2	1.1
t4038	0	0	2	2	1.1
t4206	2	0	0	2	1.1
t605	0	0	2	2	1.1
t045	0	1	0	1	0.5
t064	0	1	0	1	0.5
t11717	0	0	1	1	0.5
t1376	0	0	1	1	0.5
t13839	0	0	1	1	0.5
t17828	0	1	0	1	0.5
t17829	0	0	1	1	0.5
t17830	0	0	1	1	0.5
t17831	0	1	0	1	0.5
t17832	0	1	0	1	0.5
t1855	0	0	1	1	0.5
t1916	0	0	1	1	0.5
t1973	0	0	1	1	0.5
t214	0	0	1	1	0.5
t2395	0	0	1	1	0.5
t254	0	1	0	1	0.5
t273	0	0	1	1	0.5
t2767	0	0	1	1	0.5
t311	0	0	1	1	0.5
t3155	0	1	0	1	0.5
t3235	0	0	1	1	0.5
t335	0	0	1	1	0.5
t349	0	0	1	1	0.5
t360	0	0	1	1	0.5
t3638	0	0	1	1	0.5
t401	0	0	1	1	0.5
t4562	0	0	1	1	0.5
t458	1	0	0	1	0.5
t4938	0	0	1	1	0.5
t5468	0	0	1	1	0.5
t645	0	0	1	1	0.5
t659	0	0	1	1	0.5
t690	0	0	1	1	0.5
t7882	0	0	1	1	0.5
t8096	0	1	0	1	0.5
t934	0	0	1	1	0.5

SSTI: Skin and Soft Tissue Infection

In contrast, *S. aureus* isolates from cow milk were the least genetically diverse; i.e. only seven different *spa* types were documented where two third of the isolates had the same *spa* type. Briefly, most of the cow milk *S. aureus* isolates were *spa* type t042 (29/47, 61.7%) followed by t2856 (9/47, 19.1%), t306 (3/47, 6.4%), t085 and t4206 (2/47, 4.3% each), t5338 and t458 (1/47, 2.1% each) (Table 3.12).

The findings of the present study also showed that *spa* types t042, t306, t085, t2856 and t5338 were the only *spa* types detected from both human and dairy cows. Among these, t306 was detected in 3/47 (6.4%) of dairy cow milk isolates, in 10 of 121 (8.3%) SSTI isolates and in one of 22 (4.5%) of nasal isolates of dairy farmers. The *spa* type t085 was detected in two of the 47 cow isolates (4.3%) and in eight of the 121 SSTIs isolates (6.6%). The *spa* type t042 was detected in 29 of 47 cow isolates (61.7%), in one of 22 nasal isolates (4.5%) and in one of 121 SSTI isolates (0.8%). The *spa* type t2856 was detected in nine of 47 cow isolates (19.1%) and in one of 22 nasal isolates (4.5%). The *spa* type t5338 was found in one cow, one nasal and one SSTI isolates.

Generally, the present study showed that *S. aureus spa* type t355 was the most common genotype found in association with SSTIs in the current study area. Other *spa* types isolated from SSTIs at least 3 times included t084, t085, t127, t314, t6218, t261 and t002. *S. aureus* genotypes that were found as nasal colonizing strains of farm workers but not associated with SSTIs include t2856, t045, t064, t17828, t17831, t17832, t254, t3155 and t8096. Although not specific, *spa* types t042 and t2856 were the most cows' udder quarters adapted *S. aureus* genotypes. However, t4206 and t458 were cows' milk specific isolate although not common.

3.7. Probable clonal complex (CC)

Based on the *spa* sequence information, 178 of the 190 *S. aureus* isolates (90.5%) were assigned in to 11 probable clonal complexes (CCs) and the remaining strains had unknown CC. The identified CCs include CC1, CC5, CC8, CC15, CC22, CC25, CC80, CC88, CC121, CC152 and CC239.

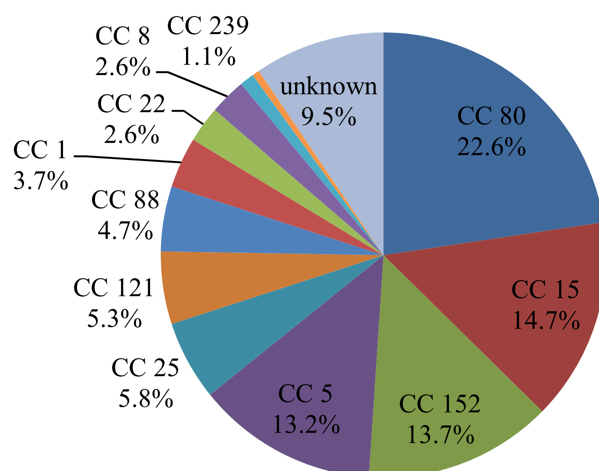


Figure 3.3: Percentage of probable CCs based on *spa* sequence of 190 *S. aureus* isolates Mekele, Northern Ethiopia

Among the 11 identified CCs, CC80 was the leading CC comprising 44 out of the 190 isolates (23.2%) followed by CC15 (28/190, 14.7%), CC152 (26/190, 13.7%), CC5 (25/190, 13.2%), CC25 (11/190, 5.8%), CC121 (10/190, 5.3%), CC88 (9/190, 4.7%), CC1 (7/190, 3.7%), CC22 (5/190, 2.6%), CC8 (5/190, 2.6%), and CC239 (2/190, 1.1%) as shown in Figure 3.3.

Table 3.13: Probable Clonal Complex of 190 *S. aureus* isolates from different sources, Mekele Northern Ethiopia

Probable CC	Source of isolate			Total	Percent
	Milk	Nasal	SSTI		
CC 80	40	2	2	44	23.2
CC 15	2	3	23	28	14.7
CC 152	0	1	25	26	13.7
CC 5	3	2	20	25	13.2
CC 25	0	1	10	11	5.8
CC 121	0	1	9	10	5.3
CC 88	0	2	7	9	4.7
CC 1	1	0	6	7	3.7
CC 22	0	2	3	5	2.6
CC 8	0	3	2	5	2.6
CC 239	0	0	2	2	1.1
Unknown CC	1	5	12	18	9.5

As shown in Table 3.13, a total of 11 CCs were observed on the 121 *S. aureus* isolates of SSTIs with known CCs. Among these, the leading CC was CC152 (25/121, 20.7%) followed by CC15 (23/121, 19%), CC5 (20/121, 16.5%), CC25 (10/121, 8.3%), CC121 (9/121, 7.4%), CC88 (7/121, 5.8%), CC1 (6/121, 5%), CC22 (3/121, 2.5%), CC8 and CC239 (2/121, 1.7% each). The 47 *S. aureus* isolates from dairy cows were assigned into 5 CCs and one isolate had unknown CC. However, most of the isolates (40/47, 85.1%) were CC80 and the rest include CC5 (3/47, 6.4%), CC15 (2/47, 4.3%), and CC1 (1/47, 2.1).

3.8. Major toxin genes possession by the top eight *spa* types

As shown in Table 3.14 the top eight *spa* types detected five or more times in this study were; t042, t355, t306, t084, t085, t2856, t127 and t314. Among these all the *spa* type t085, t314 and t355 and 83.3% of *spa* type t127 were positive for *pvl*. Only *spa* type t306 was found positive for *tsst-1*. Regarding the major classical enterotoxin genes (*sea, seb, sec,* and *sed*), 100% of *spa* type t085 and 83.3% of *spa* type t127 were positive for *sea*; 100% of t314 and 85.7% of t306 possessed *seb*; 85.7% of t306 possessed *sec* and 78.6% of t306 possessed *sed* (Table 3.14).

Table 3.14: Comparison of *spa* types with the major toxin genes of *S. aureus* isolates from Mekele, Northern Ethiopia

***Spa* type**	**Toxin Genes**											
	pvl		*sea*		*seb*		*sec*		*sed*		*tsst-1*	
	No	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
t042	31 100.0%	0 0.0%	31 100.0%	0 0.0%	31 100.0%	0 0.0%	31 100.0%	0 0.0%	31 100.0%	0 0.0%	31 100.0%	0 0.0%
t084	9 90.0%	1 10.0%	9 90.0%	1 10.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%
t085	0 0.0%	10 100.0%	0 0.0%	10 100.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%
t127	1 16.7%	5 83.3%	1 16.7%	5 83.3%	6 100.0%	0 0.0%	6 100.0%	0 0.0%	6 100.0%	0 0.0%	6 100.0%	0 0.0%
t2856	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%	10 100.0%	0 0.0%
t306	13 92.9%	1 7.1%	10 71.4%	4 28.6%	2 14.3%	12 85.7%	2 14.3%	12 85.7%	3 21.4%	11 78.6%	5 35.7%	9 64.3%
t314	0 0.0%	5 100.0%	5 100.0%	0 0.0%	0 0.0%	5 100.0%	3 60.0%	2 40.0%	5 100.0%	0 0.0%	5 100.0%	0 0.0%
t355	0 0.0%	24 100.0%	24 100.0%	0 0.0%	24 100.0%	0 0.0%	19 79.2%	5 20.8%	24 100.0%	0 0.0%	24 100.0%	0 0.0%
Total	64 58.2%	46 41.8%	90 81.8%	20 18.2%	93 84.5%	17 15.5%	91 82.7%	19 17.3%	99 90.0%	11 10.0%	101 91.8%	9 8.2%

CHAPTER FOUR: DISCUSSION

Staphylococcus aureus poses a serious concern globally due to its ability to cause a number of life-threatening diseases and continuous evolvement of drug resistance [Paterson *et al.*, 2014]. Especially, the emergence and spread of MRSA initially in the healthcare setting [Barber, 1961], later in the community [Udo *et al.*, 1993] and recently in the veterinary aspect [Fitzgerald, 2012] is a serious global threat.

In Ethiopia, previous studies have well documented *S. aureus* as a common pathogen in both human [Kahsay *et al.*, 2014; Mama *et al.*, 2014; Mengesha *et al.*, 2014; Mohammed *et al.*, 2017] and dairy cows [Abera *et al.*, 2012; Mekonnen *et al.*, 2017; Seyoum *et al.*, 2018]. However, almost all of these studies were restricted to phenotypic characterization of the bacterium. Molecular techniques are recommended for proper *S. aureus* identification, detection of specific virulence and drug resistance genes and to study the spread and distribution of specific strains [Price *et al.*, 2013]. To the best of our knowledge, there have been few molecular studies done in Ethiopia on *S. aureus* isolates from raw and processed milk [Mekonnen *et al.*, 2018; Tarekgne *et al.*, 2016; Tigabu *et al.*, 2015; Zenebe *et al.*, 2018]. As far as we know, no molecular characterization study was conducted so far on human clinical *S. aureus* isolates in Ethiopia. Therefore, the present study was conducted to isolate *S. aureus* from human skin and soft tissue infections, milk of dairy cows and nares of dairy farm workers; and to determine the antimicrobial resistance pattern, virulence genes profile and genotypic diversity of the *S. aureus* isolates.

In the present study, a total of 811 non-duplicate specimens were collected from 355 study participants suffering from SSTIs (wound culture), 71 farm workers (nasal screening) and 384 dairy cows (milk specimen). Of the 355 study participants with SSTI, 61% were with clinically suspected community onset and 39% with suspected surgical site infections (hospital acquired). Their age ranged from 3 months to 90 years with mean age of 30.5 years. Majority were males (61.7%), from urban area (62.5%) and were from the dermatology department (42%) followed by adult surgical ward (30.7%). The 384 dairy cows were selected from 67 dairy farms located in Mekele, Northern Ethiopia. Their age

ranged from 3-14 years with the mean age of 5.9 years. Majority of them (66%) gave birth to 1-3 calves. More than half (52.7%) had less than three months of lactational stage and their milking process was all by manual means. From the 67 dairy farms, 71 farmers participated in the study and out of these, 77.5% were males and their aged ranged from 17 to 63 years old.

S. aureus is a common cause of skin and soft tissue infections (SSTIs) both in the community and healthcare setting [Bassetti *et al.*, 2017]. The major SSTIs caused by *S. aureus* include folliculitis, furuncles, abscesses, impetigo, carbuncles, cellulitis, and surgical site infections [Bouchiat *et al.*, 2017]. Severe SSTIs can progress to deep infections; such as bone, joints, the heart and other internal organs; leading to increased morbidity and mortality [Macmorran *et al.*, 2017; Watanabe *et al.*, 2017]. The prevalence of *S. aureus* is higher in community acquired compared to health care associated skin and soft tissue infections. This is because the etiologies of HA-SSTI, mainly surgical site infection, are diverse compared to community onset SSTIs which are mainly due to *S. aureus* and *S. pyogenes* [Yamamoto, 2017]. In addition, *S. aureus* strains that cause community associated SSTIs are more virulent than those that cause health care associated SSTIs. This is because community associated *S. aureus* strains possess more virulence genes, such as *pvl* leading to higher virulence, that cause infections in healthy individuals unlike those strains causing health care associated infections, which usually require predisposing factors [Bassetti *et al.*, 2017].

In the present study, *S. aureus* was isolated from 34.6% of study participants with SSTIs and the isolation rate of *S. aureus* was higher in community onset SSTIs (40.5%) compared to surgical site infections (25.4%). This is a comparable finding with previous studies conducted in Ethiopia which reported *S. aureus* isolation rate of 31.3% in SSTIs, Jimma University Hospital, Southwest Ethiopia [Mama *et al.*, 2014] and 28.5% in SSTIs, Gondar University Hospital, Northwest Ethiopia [Mohammed *et al.*, 2017].

In the present study, *S. aureus* was isolated from 40.5% of study participants with community onset SSTIs. A comparable finding was reported from Indonesia where 45.3% of community onset-SSTIs were due to *S. aureus* [Santosaningsih *et al.*, 2018]. In contrast,

higher percentage of *S. aureus* isolation from CA-SSTIs was reported in USA (88%) [Magilner *et al*., 2008], China (71.6%) [Liu *et al.*, 2016b], Spain (66%) [Casado-Verrier *et al.*, 2012] and United Arab Emirates (62%) [Al Jalaf *et al.*, 2018]. A slightly lower percentage of *S. aureus* isolation from CO-SSTIs was reported from Greece (31%) [Vourli *et al.*, 2009] compared to the present finding in Ethiopia (40.5%). Despite variations in percentage of isolation of *S. aureus* in different countries, all studies indicated that *S. aureus* is a significant cause of community onset-SSTIs.

In the present study, *S. aureus* was isolated from 25.4% of patients with clinical signs of surgical site infections. This is comparable with a study from Uganda where 28.7% of patients with SSI were positive for *S. aureus*. However, previous studies conducted in Ethiopia reported higher *S. aureus* prevalence in SSIs that ranged from 34.4% to 39.7% [Kahsay *et al.*, 2014; Mengesha *et al.*, 2014]. Infection prevention practices may contribute to variations in the frequency of isolation of *S. aureus* from surgical site infections in different hospitals.

S. aureus exist as a commensal in different body parts of human, animals and the environment. The major colonization sites for *S. aureus* are the nares. Colonization is believed to provide a reservoir for infection when host defenses are breached and allows the bacterium to be transmitted among humans, animals and between them [Gordon and Lowy, 2008]. *S. aureus* cross-transmission between human and animals was mainly demonstrated among dairy farm workers due to their close contact with animals [Smith, 2015]. Global estimates showed that about a third of world population carry *S. aureus* in their nares.

In the present study, 31% of dairy farmers were found colonized by *S. aureus* in their nares which is higher than a previous study done around Addis Ababa, Ethiopia where 13.2% of dairy farmers were found colonized [Mekuria *et al.*, 2013]. In the present study, tryptic soy broth as an enrichment media was used to maximize the recovery of *S. aureus* from nasal swabs and this might explain the higher frequency of isolation as compared to the similar study conducted in Ethiopia [Mekuria *et al.*, 2013]. Studies from other parts of the world also showed a significant nasal carriage of *S. aureus* from farm workers; 15.2% of dairy

farm workers carried *S. aureus* in their nares in South Africa [Schmidt *et al.*, 2015] and 36% in Catania, Italy [Antoci *et al.*, 2013].

S. aureus is also one of the most common bacteria capable of infecting udder quarters of dairy cows. Infected dairy cows can shed large amount of bacteria in their milk. Consumption of such contaminated milk can lead to human infections or intoxications if the *S. aureus* strain excretes enterotoxins [De Vliegher *et al.*, 2012; Sartori *et al.*, 2018]. Besides the risk of human infection, udder infections can have a direct effect on the welfare of the animal and economic loss to farmers due to decreased milk production or abnormality of the milk [Peton and Le Loir, 2014]. Several studies in Ethiopia and elsewhere have documented *S. aureus* as an important bacterium of dairy animals' intra-mammary gland infection. There is strong research based evidence showing *S. aureus* as an important pathogen in the dairy industry both in Ethiopia and elsewhere [Abebe *et al.*, 2016; Ericsson Unnerstad *et al.*, 2009; Mekonnen *et al.*, 2018; Mekuria *et al.*, 2013].

In the present study, milk samples from 12.5% of dairy cows were found to be positive for *S. aureus*. Similarly, previous studies in Ethiopia also reported an *S. aureus* prevalence of 9% to 27.9% [Abebe *et al.*, 2016; Mekonnen *et al.*, 2017; Mekuria *et al.*, 2013; Seyoum *et al.*, 2018]. Other studies in Western Zambia [Knight-Jones *et al.*, 2016], Zimbabwe [Katsande *et al.*, 2013] and Northern Italy [Riva *et al.*, 2015] have reported an *S. aureus* isolation rate of 22%, 16.3% and 9.1%, respectively from cows' milk.

Antimicrobial resistance poses a serious threat to both human and animal health. The overuse and misuse of antimicrobials is accelerating the emergence of drug resistant bacteria. The use of antimicrobials in food-producing animals either for treatment or for growth promotion can lead to selection and dissemination of antimicrobial-resistant bacteria in these animals, which can then be transmitted to humans via food and other transmission routes [Santy-Tomlinson, 2018; WHO, 2017]. *S. aureus* is one of these bacteria affecting both human and animals. It is in a continuous evolution of drug resistance since the introduction of the first antibiotic (penicillin) in 1941. Penicillin resistance by *S. aureus* was reported two years after the introduction of the drug, initially in the health care setting and

later in the community [Deurenberg and Stobberingh, 2008]. Since then, penicillin resistant *S. aureus* has increased and spread widely and majority of isolates are resistant to penicillin nowadays [Malachowa and DeLeo, 2010].

In the present study, 92.7% of overall *S. aureus* isolates were resistant to penicillin. Resistance to penicillin was observed in 92% of community onset SSTIs isolates, 97.1% of surgical site infection isolates, 91.7% of cows' milk isolates and 90.9% of dairy farmers isolates. Similar findings have been reported in previous studies conducted in Ethiopia e.g. 82-91.5% penicillin resistance was documented on human *S. aureus* isolates from SSTIs [Kahsay *et al.*, 2014; Mama *et al.*, 2014; Mohammed *et al.*, 2017] and 97-100% isolates from milk of dairy cows in Ethiopia [Mekuria *et al.*, 2013; Seyoum *et al.*, 2018]. High percentage of penicillin resistant *S. aureus* was also documented in a multicenter study on patients with SSTIs in China (96.6%) [Liu *et al.*, 2016b], patients with SSTIs and bone related infections in Benin (100%) [Sina *et al.*, 2013] and on bovine milk isolates in Egypt (83.3%) [Awad *et al.*, 2017].

To overcome infections due to penicillin resistant *S. aureus*, β-lactamase resistant penicillin namely methicillin was introduced in 1959. However, the victory against *S. aureus* using methicillin was short-lived and the first methicillin-resistant *S. aureus* (MRSA) strain was identified in 1961 in a hospital [Barber, 1961]. Shortly thereafter, MRSA became pandemic in many healthcare institutions worldwide [Waness, 2010]. In addition, MRSA started to appear in the community who lack risk factors for exposure to the health care systems [Udo *et al.*, 1993]. Since the first report, there has been an explosion in the number of CO-MRSA infections worldwide [David and Daum, 2010]. In addition, the emergence of MRSA in animals capable of being transmitted to human in recent years poses a serious health threat [Fitzgerald, 2012]. The distribution of MRSA seems to vary by geographic location and diffuses more in humans than animals.

In the present study, *S. aureus* harbouring *mecA* (MRSA) are reported in 2.4% of SSTIs isolates which is much lower than most reports obtained from different parts of the world. In North America, MRSA accounted for up to 85% of *S. aureus* isolates from CO-SSTIs in the

USA [Magilner *et al.*, 2008; Mistry *et al.*, 2014] and up to 56% in Canada [Muileboom *et al.*, 2013; Stenstrom *et al.*, 2009]. In Europe, MRSA accounted for 0% -29% of SSTI *S. aureus* isolates in a multi-center study in seven European countries [Bouchiat *et al.*, 2017], 33% in Spain [Casado-Verrier *et al.*, 2012] and 30.7% in Greece [Vourli *et al.*, 2009]. In Australia, 60% of *S. aureus* isolates from community onset SSTIs were MRSA [Macmorran *et al.*, 2017]. In Asia, the prevalence of MRSA among *S. aureus* isolates from SSTIs was 57.7% in Taiwan [Changchien *et al.*, 2016], 29% in United Arab Emirates [Al Jalaf *et al.*, 2018], 24.4% in Japan [Watanabe *et al.*, 2017] and 2.6% in China [Liu *et al.*, 2016b]. In Africa, the prevalence of MRSA was as high as 84% from SSTIs in Kenya [Maina *et al.*, 2013], 31.5% in Uganda [Ojulong *et al.*, 2009], 25% in Benin [Sina *et al.*, 2013], 22.6% in Botswana [Truong *et al.*, 2011] and 1.3% of CO-SSTI isolates in Mozambique [van der Meeren *et al.*, 2014]..

In the present study, no *mecA/mecC* positive *S. aureus* was detected from bovine milk. Similar findings have been reported in the previous study conducted in central Ethiopia [Tigabu *et al.*, 2015], in Tunisia [Ben Said *et al.*, 2016] and Australia [Worthing *et al.*, 2018]. In contrast, up to 52% of *S. aureus* isolates from milk of dairy cows were positive for MRSA in Egypt [Awad *et al.*, 2017; Elhaig and Selim, 2014], 17% in Turkey [Turkyilmaz *et al.*, 2010], 11% in Brazil [Silva *et al.*, 2014], 9.3% in Belgium [Vanderhaeghen *et al.*, 2010], 6.2% in Korea [Nam *et al.*, 2011]. Findings of the present and the previous studies indicate that no MRSA is circulating in dairy cows in Ethiopia.

Vancomycin became the drug of choice for the treatment of infections caused by MRSA [Cole and Riordan, 2013]. However the success of treatment wasn't long lived in which the first case of vancomycin-intermediate *S. aureus* (VISA) [Hiramatsu *et al.*, 1997] and vancomycin-resistant *Staphylococcus aureus* (VRSA) [Sievert *et al.*, 2008] were reported in 1997 and 2002, respectively. VISA (MIC, 4-8 μg/ml) is due to thickening of the *S. aureus* cell wall but VRSA (MIC ≥16 μg/ml) is due to possession of the *vanA* by the bacterium. Intermediate resistance is more common than high resistance [Deresinski, 2013]. Since the first report, VRSA has been also reported from India [Thati *et al.*, 2011], Iran [Azimian *et al.*, 2012] and Pakistan [Hakim *et al.*, 2007].

In the present study, vancomycin resistance was not observed in both human and dairy cows' *S. aureus* isolates. This is in agreement with previous studies conducted in Ethiopia [Mama *et al.*, 2014; Mengesha *et al.*, 2014; Mohammed *et al.*, 2017; Seyoum *et al.*, 2018; Tigabu *et al.*, 2015], Kenya [Maina *et al.*, 2013], Benin [Sina *et al.*, 2013], Mozambique [van der Meeren *et al.*, 2014], Nigeria [Ghebremedhin *et al.*, 2009], Uganda [Ojulong *et al.*, 2009], 23 hospitals in China [Liu *et al.*, 2016b] and Taiwan [Changchien *et al.*, 2016]. In contrast, some previously conducted studies in Ethiopia reported vancomycin resistance of 4% among *S. aureus* isolates from SSI [Kahsay *et al.*, 2014] and 2.4% from milk of dairy cows [Mekuria *et al.*, 2013]. In the present study, susceptibility testing for vancomycin and Daptomycin was done using the E-test method to determine the minimum inhibitory concentration (MIC), whereas the previous studies in Ethiopia employed disc diffusion method which is not recommended for Vancomycin susceptibility testing [CLSI, 2014]. Variation in the methodology of susceptibility testing for Daptomycin and vancomycin can affect the interpretation of the susceptibility testing result. The research findings in Ethiopia collectively indicated the absence of vancomycin resistant *S. aureus* (VRSA) is the country despite its use.

The overall resistance of *S. aureus* to the other tested antimicrobial agents in this study was 38.3% for Tetracycline, 31.6% for Trimethoprim-Sulphamethoxazole, 18.7% for Erythromycin, 5.2% for clindamycin and no resistance was observed against Daptomycin and Rifampin. No statistical significant difference was observed on the resistance pattern between human and dairy cows' isolates except for Erythromycin (25.2% vs. 2.1%, $p=0.002$) and Trimethoprim-Sulphamethoxazole (40.7% vs. 10.4%, $p=0.001$). This might be because of frequent use of both antibiotics in humans as prophylaxis or treatment than in animals and this creates selective pressure on the strains and selecting the emergence of drug resistant strains.

The present study also documented that 94.8% of the overall *S. aureus* isolates were resistant to at least one of the nine antimicrobial agents (classes) tested and 25.9% were multi-drug resistant (resistant to three or more antimicrobial classes). However, MDR was significantly higher ($p<0.001$) among human clinical isolates (35.8%) compared to the nasal

colonizing isolates (13.6%) and cows' milk isolates (6.3%). Dekker *et al*. (2016) from Ghana reported similar MDR finding on human clinical *S. aureus* isolates (35.7%) [Dekker *et al.*, 2016]. A study from Egypt showed that 83.3% *S. aureus* isolates from dairy cows were MDR [Awad *et al.*, 2017] which is in contrast with the findings of the present study where 6.3% of milk isolates were MDR.

S. aureus is known to secrete a number of toxins which play a major role during disease pathogenesis. These toxins are broadly classified as pore-forming toxins which include the Panton-Valentine leukocidin (PVL), exfoliative toxins and superantigens (SAgs). More than 23 SAg toxins have been described, including toxic shock syndrome toxin (TSST-1), staphylococcal enterotoxins (SEA to SEE, SEG to SEJ, SEL to SEQ and SER to SET), and 11 staphylococcal superantigen-like toxins (SEIK to SEIQ, SEIU to SEIX) [Oliveira *et al.*, 2018].

The present study targeted 11 of these toxin coding genes; i.e. *pvl, tsst-1* and nine staphylococcal enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei* and *sej*). As a result, 67% of the overall *S. aureus* isolates were found harboring at least 1 of the 11 toxin genes. There was a statistically significant difference on the possession of these toxin genes among human, nasal and cows' milk isolates which was 81.3%, 63.6% and 31.2%, respectively ($P<0.001$). Among the 11 toxin genes *pvl* was the leading gene harbored by 36.8% of the isolates. This toxin gene was also the most frequently identified gene in 53.7% human clinical isolates whereas *seb* and *seh* were the dominant genes detected in 22.7% nasal colonizing isolates and 16.7% dairy cows' milk isolates.

The Panton-Valentine leukocidin (PVL) toxin is one of the important toxins in which *S. aureus* strains could possess. It causes lysis of polymorphonuclear leukocytes, monocytes and macrophages when it binds to complement receptors on their membrane [Saeed *et al.*, 2018; Shallcross *et al.*, 2013]. However, not all *S. aureus* strains possess *pvl*. A recent meta-analysis on 76 studies from 31 countries concluded that *pvl* producing *S. aureus* strains are strongly associated with skin and soft-tissue infections, but are comparatively rare in pneumonia, bacteremia, and those colonizing other sites [Shallcross *et al.*, 2013]. In

addition, several studies elsewhere also reported that *S. aureus* strains isolated from milk of dairy cows are less likely to possess *pvl* [Asiimwe *et al.*, 2017b; Awad *et al.*, 2017; Ben Said *et al.*, 2016; Riva *et al.*, 2015]. In the present study, similar finding has been reported for the first time in Ethiopia that *pvl* is most frequently detected on *S. aureus* isolates from SSTIs (53.7%) compared to nasal colonizing (13.6%) and milk isolates (4.2%).

The present investigation also noted that the proportion of *pvl* positive strains was higher in isolates from community onset (44.3%) than hospital onset (42.9%) SSTIs. Similar findings have been reported from Taiwan [Changchien *et al.*, 2016], Mozambique [van der Meeren *et al.*, 2014], China [Yao *et al.*, 2010] and Colombia [Portillo *et al.*, 2013]. Although studies from different continents showed a strong link between *pvl* and SSTIs, its proportion seems to vary by geographic location; for example, as high as 80% of *S. aureus* were found positive for *pvl* in Nigeria [Ayepola *et al.*, 2015], 73% in the USA [Pardos de la Gandara *et al.*, 2015], 65% in Colombia [Portillo *et al.*, 2013], 49.3% in Uganda [Asiimwe *et al.*, 2017a], 23.4% in China [Yao *et al.*, 2010], 20.3% in Kenya [Maina *et al.*, 2013], 2.5% in Turkey [Gulmez *et al.*, 2012] and as low as 0.7% in Spain [Marimon *et al.*, 2012].

In relation to *pvl*, the present study found only one of the four MRSA isolates was positive for *pvl*. This strain was isolated from a patient with community onset SSTI and harbored SCC*mec* IV. The remaining three MRSA (2 MRSA strains with SCC*mec* III and one MRSA strain with SCC*mec* IV) were *pvl* negative. This is in agreement with studies done elsewhere, where *pvl* was found mainly in association with MRSA strains harboring SCC*mec* IV, a strain highly linked with CO-SSTIs [Changchien *et al.*, 2016; O'Hara *et al.*, 2012; Vourli *et al.*, 2009]. Definite conclusion could not be reached with few numbers of MRSA to explain the association between *pvl* with MRSA strains harboring SCC*mec* IV unless large numbers of MRSA are screened for *pvl* and SCC*mec* IV.

With regard to the superantigen toxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej* and *tsst-1*), 56% of the overall *S. aureus* isolates were found to harbor at least one of the 10 superantigen genes. The present finding (56%) is comparable with the earlier finding in Northern Ethiopia (51%) [Tarekgne *et al.*, 2016] but lower than findings reported in Uganda

(90.2%) [Asiimwe *et al.*, 2017b], Moroco (76%) [Elazhari *et al.*, 2011] and Brazil (65.8%) [Costa *et al.*, 2018]. The present study also found at least one classical enterotoxin gene (*sea, seb, sec, sed, see*) in 38.3% of the overall *S. aureus* isolates. However, human clinical isolates from SSTIs seemed to carry one of these genes more frequently (48.8%) compared to the nasal (36.4%) and cows' milk (12.5%) isolates (*p<0.001*). Tarekgn *et al* (2016) found at least one of these genes in 13.75% of *S. aureus* milk isolates in Northern Ethiopia which is comparable finding with the present study (12.5%). In contrast, higher percentage of these genes was reported from milk isolates in central Ethiopia (67%) [Seyoum *et al.*, 2016] and in Italy (45.7%) [Riva *et al.*, 2015].

In this study, among the nine enterotoxin genes, *seg* and *sei* were the most frequently detected (each of them detected in 29% of total isolates). This in agreement with the previous studies conducted in Northern Ethiopia [Tarekgne *et al.*, 2016], Uganda [Asiimwe *et al.*, 2017b], Colombia [Portillo *et al.*, 2013] and China [He *et al.*, 2018]. The *seg* and *sei* also showed highest gene combination which existed all together. The same pattern of gene combination observed from previous study conducted in Ethiopia [Tarekgne *et al.*, 2016]. The scientific explanation for this is that *seg* and *sei* are part of the enterotoxin gene cluster (egc) of *S. aureus* along with *sem, sen,* and *seo* [Portillo *et al.*, 2013]. In addition, this study found *seg* and *sei* significantly higher in *S. aureus* isolates from skin and soft tissue infections and nares of farm workers compared to cows' milk isolates (*p=0.001*). However, the association of *seg* and *sei* positive *S. aureus* strains with human infection and colonization needs further investigation.

The *seb* was the 3rd most frequently detected from the overall enterotoxin genes tested and the leading among the classical enterotoxin genes and followed by *sea* and *sec;* each of them detected in 19.2%, 16% and 15% of isolates, respectively. He *et al*. (2018) from China also reported *seb* as the most frequently detected gene followed by *sea*, each detected in 22.5% and 21.3% respectively [He *et al.*, 2018]. Similar studies also demonstrated *seb* as the most common enterotoxin gene detected in 44% and 18.6% of *S. aureus* clinical isolates from human in Benin [Sina *et al.*, 2013] and Colombia [Vanegas Munera *et al.*, 2017], respectively.

The *sea* was the leading classical enterotoxin gene among the cows' milk isolates and detected in 10.4% of the isolates in the present study. This is in agreement with previous studies conducted in Ethiopia that showed *sea* as the most common enterotoxin gene from *S. aureus* milk isolates [Seyoum *et al.*, 2016; Tarekgne *et al.*, 2016]. However, studies from Uganda and South Africa reported *sec* as the leading classical enterotoxin gene among bovine milk isolates, respectively [Asiimwe *et al.*, 2017b]. The present study along with the two previous Ethiopian studies [Seyoum *et al.*, 2016; Tarekgne *et al.*, 2016] suggested that *seb* and *sea* positive *S. aureus* strains could be the predominant strains from human SSTIs and cows' intramammary gland infection, respectively.

In the present study, no *see* was detected and the percentage of *sed* was low (8.3%). This is in agreement with findings reported from Nigeria [Ayeni *et al.,* 2018] and Brazil [Arcuri *et al.,* 2010] where none of *S. aureus* clinical isolates from human and dairy products harbored *see,* respectively. However, studies from Colombia [Vanegas Munera *et al.*, 2017] and Benin [Sina *et al.*, 2013] were able to detect *see* but still in a very low frequency (<1% of isolates). The finding of the present study either no or very low frequency of *sed* among *S. aureus* strains is consistent with previous findings reported in Ethiopia and elsewhere [Ghebremedhin *et al.*, 2009; Sina *et al.*, 2013; Vanegas Munera *et al.*, 2017]. In contrast, the previous study in central Ethiopia reported *see* in 16.5% of bovine milk isolates and was the 3rd most common of the classical enterotoxin genes [Seyoum *et al.*, 2016]. The variation of *see* or *sed* detection from the present study and other studies conducted in Ethiopia could be due to a difference in the geographic location of the study areas which might indicate that *see* positive strains could be circulating in the bovine population in central Ethiopia but not in Northern part of the country.

The present study documented *tsst-1* in 9.8% of the overall *S. aureus* isolates and all of them were from human isolates. Specifically, 12% of the human clinical isolates and 18.2% of nasal isolates were positive for this gene. It has been well demonstrated that *tsst-1* positive *S. aureus* strains are spreading in the human population, although the proportion varies from area to area. For example, the proportion of *tsst-1* positive *S. aureus* strains ranges from 0% in Italy to 68% in Iran [Antoci *et al.*, 2013; Sina *et al.*, 2013; Wang *et al.*, 2017; Zarei

Koosha *et al.*, 2016]. Similar to the present study, no *tsst-1* was found in dairy cows' milk in Tunisia [Ben Said *et al.*, 2016]. However, the two previous studies in Ethiopia reported *tsst-1* in 15% and 47% dairy products, respectively [Seyoum *et al.*, 2016; Tarekgne *et al.*, 2016]. This variation may result from the difference in the sample collection procedure. In the present study, milk samples were directly collected from udder quarters to sterile containers whereas Seyoum *et al.* (2016) collected on-farm pooled and combined bulk tank milk and Tarekgne *et al.* (2016) collected bulk milk and milk product samples which could possibly be contaminated by human *S. aureus* strains either during processing or from the containers in case of the bulk milk samples.

The *tsst-1* positive *S. aureus* isolates in the present study were not only human restricted but also clonally related. Most of them fall in the CC5 (66.7%) and CC22 (27.7%). Consistent with the present study, *tsst-1* was detected predominantly in CC5 up to 96.3% in China [He *et al.*, 2018; Wang *et al.*, 2017]. The study by Wang *et al*. (2017) also showed that *tsst-1*-positive CC5 strains were found associated with higher mortality. Hence, the existence of *tsst-1*-positive CC5 strains in the present study warrants better infection prevention practices should be in place in the study area.

spa typing is one of *S. aureus* typing tools and characterized by its excellent discriminatory power, ease of performance, cheaper procedure, and standardized nomenclature [Asadollahi *et al.*, 2018]. To date there are more than 17897 *S. aureus spa* types from 135 countries as indicated in the SpaServer Database (https://www.spaserver.ridom.de/).

In the present study, 98.4% *S. aureus* isolates were successfully typed using the Ridom *spa* typing method. This resulted in 61 different *spa* types and out of these, five were novel and all of them were originated from human; i.e. *spa* types t17828, t17829, t17830, t17831 and t17832. This is the first report in Ethiopia. Of the overall 61 different *spa* types identified, t042 (16.3%) was the most common followed by t355 (12.6%) and t306 (7.4%). However, there was variation on *spa* types between human and cows' isolates. Among the human clinical isolates, t355 was the most dominant (19%) and followed by t306 (8.3%), t084 and t085 (6.6% each), t127 (5%) and t314 (4.1%). Similar findings have been reported in two

different studies on human clinical *S. aureus* isolates from Ghana showed t355 as the most common *spa* type [Dekker *et al.*, 2016; Egyir *et al.*, 2014]. Egyir *et al*. (2014) found t355 in 19% of isolates followed by t084 (12%) and t314 (6%). Similarly, Dekker *et al.* (2016) found t355 in 19.6% followed by t084 and t314 (each account for 14.3%) and t311 (8.9%). In contrast, t084 was the most common *spa* type in Nigeria [Ayepola *et al.*, 2015] and China [Wu *et al.*, 2010], t891 (13.9%) in South Africa [Oosthuysen *et al.*, 2014] and t003 in Russia [Baranovich *et al.*, 2010].

S. aureus isolates from milk of dairy cows (only 7 different *spa* types) were less diversified compared to human isolates where 50 different *spa* types of SSTI isolates and 19 different *spa* types of nasal isolates that are highly diversified. Similarly, a recent study from South Africa also reported bovine isolates were genetically less diversified compared to human *S. aureus* isolates [Schmidt *et al.*, 2017]. In the present study, the *spa* type t042 was the most dominant accounting for two third (61.7%) of milk isolates from dairy cows followed by t2856 (19.1%), t306 (6.4%), t085 and t4206 (2/47, 4.3% each). This is inagreement with the recent study in North-Western Ethiopia where t042 was the most frequent spa type accounting for 58% of bovine quarter milk isolates [Mekonnen *et al.*, 2018]. However, the previous study on bulk milk and milk products in Northern Ethiopia found t314 as the most frequent *spa* type (20.7%) followed by t458 (18.3%) and t6218 (9.8%) [Tarekgne *et al.*, 2016]. In the present study, t458 was detected from one isolate which was from milk; however, no milk isolate was either t314 or t6218. Interestingly, five isolates with *spa* type t314 and four with *spa* type t6218 in this study were human clinical isolates. It is therefore possible that *S. aureus spa* type t314 and t6218 are human strains and their existence in the bulk milk and milk product samples in the previous study might be due to human contamination. Like the human clinical isolates, the dominant *S. aureus spa* types from milk vary from location to location. For example, t2421 was the leading *spa* type in Tunisia [Ben Said *et al.*, 2016], t7753 and t1398 in Uganda [Asiimwe *et al.*, 2017b] and t127 in Brazil [Silva *et al.*, 2013].

In the present study, *S. aureus* isolates of nasal origin were the most genetically diverse where 19 different *spa* types were documented from 22 isolates. However, no predominant *spa* type could be identified from these nasal colonizing strains.

The *spa* types common to both human and dairy cows in this study included t042, t306, t085, t2856 and t5338. Among theses, t306 seems to be the most identified *spa* type in both human and animals detected in 6.4% of dairy cows' isolates, 8.3% of human clinical isolates and 4.5% of nasal colonizing isolates of dairy farmers. The *spa* type t085 was detected in 4.3% of cows' isolates and 6.6% human clinical isolates. *spa* t042, which was the most frequent *spa* type among the cows' isolates, also isolated from nare of dairy farm workers (4.5% of isolates) and from human SSTIs (0.8% of isolates). The *spa* t2856 constituted 19.1% of the cows' isolates and 4.5% of nasal isolates but not detected from SSTIs. The *spa* type t5338 was found in one cow, one nasal and one SSTI isolates. Collectively, these *spa* typing data indicated that there are some *S. aureus* strains adapted to both human and dairy cows in the study area particularly *spa* t306 and t085 which were capable of causing human SSTIs and exist in cows' udder quarters.

Based on the *spa* sequence information, 90.5% of the total *S. aureus* were assigned into 12 probable clonal complexes (CCs). The identified CCs include CC1, CC5, CC8, CC15, CC22, CC25, CC80, CC81, CC88, CC121, CC152 and CC239. The leading CC among the human clinical isolates was CC152 (20.7%) followed by CC15 (19%) and CC5 (16.5%). This is in agreement with a previous study on SSTIs isolates from asylum seekers from Eritrea and individuals in contact with them in Switzerland reported CC152 and CC15 strains cause most SSTIs infections [Jaton *et al.*, 2016]. This indicates that CC152 and CC15 strains are highly spread in the two neighboring countries and possibly eastern Africa. Similarly, the two studies from Ghana also reported CC152 and CC15 as the most common CCs in human clinical isolates [Dekker *et al.*, 2016; Egyir *et al.*, 2014]. In contrast, the most common clonal complex among human clinical isolates was CC8 followed by CC5 in Colombia [Vanegas Munera *et al.*, 2017] and CC891 in South Africa [Oosthuysen *et al.*, 2014]. The present study also found that half of the MRSA isolates were CC239, which is globally spread.

In the present study, most of the *S. aureus* isolates from dairy cows (83%) were CC80 followed by CC5 (6.4%), CC15 (4.3%), CC1 and CC81 (2.1% each). This in contrast, CC5 was the predominant clonal complex among milk isolates in Kenya [Omuse *et al.*, 2016]; CC97 followed by CC1 and CC15 in Tunisia [Ben Said *et al.*, 2016]; CC398, CC97, and CC1 in Italy [Locatelli *et al.*, 2017] CC1 and CC126 in Brazil [Silva *et al.*, 2013].

LIMITATIONS OF THE STUDY

- Chromogenic media was not used for screening MRSA; hence, some MRSA strains might be missed or passed undetected.
- MLST was not performed on selected isolates because of budget constraints

CONCLUSIONS

In the present study, *S. aureus* was isolated from 34.6% of skin and soft tissue infections, 31% of nares of farm workers and 12.5% of milk of dairy cows in Mekele, Northern Ethiopia. Highest resistance was observed for Penicillin (92.7%), but no resistance was observed for Vancomycin, Daptomycin and Rifampin. This study reports *mecA* positive *S. aureus* (MRSA) in 2.1% of the isolates, in which all of them were of human origin. Human clinical isolates were more multi-drug resistant (35.8%) compared to nasal colonizing isolates (13.6%) and cows' milk isolates (6.3%). More than 80% of human clinical isolates harbored at least one of the 11 genes tested as compared to 31% in cows' milk isolates. Among the toxin genes, *pvl* was the most common gene harbored by 36.8% of the overall isolates and was found significantly higher on SSTIs isolates (53.7%) compared to nasal (13.6%) and milk (4.2%) isolates. Of the superantigen genes, *seg* and *sei* were the most frequently detected (29%) followed by *seb* (19%), *sea* (16%) and *sec* (15%). No *see* positive *S. aureus* was found in this study. The *tsst-1* was detected from 12% of SSTIs and 18.2% of nasal isolates but not from milk. Two third of the *tsst-1* positive strains were CC5. Overall, *S. aureus* isolates showed high genetic diversity where 61 different *spa* types were detected grouped in 12 CCs. However, dairy cows isolates were less diversified compared to human isolates. Among the SSTI isolates, t355 was the most dominant (19%) followed by t306

(8.3%), t084 and t085 (6.6% each). On the other hand, t042 was the most common accounting for two third of the milk isolates followed by t2856 (19.1%) and t306 (6.4%). The *spa* type t042, t306, t085, t2856 and t5338 were common to both human and dairy cows indicating a possibility of human-animal cross-transmission in the study area. The most common clonal complex among the SSTI isolates was CC152 whereas CC80 among the milk isolates. *S. aureus* strains of CC152 and CC121 were all *pvl*-positive.

RECOMMENDATIONS

The following recommendations were made based on the findings of the present study:

- Findings of the present study indicate that *S. aureus* is a significant cause of skin and soft tissue infections in human and intramammary infections in dairy cows. Hence, measures to reduce/prevent infection such as improving infection prevention practices in the hospital, keeping hygiene of the dairy cows and educating the community to practice the best possible personal hygiene are recommended.
- The high prevalence of penicillin resistance but low MRSA prevalence in this study suggests that antibiotics in the Oxacillin group can still be recommended for the treatment of Skin and soft tissue infections in the study area. However, the detection of multidrug resistant *S. aureus*, such as the MRSA strains of the CC239 which are spread globally, warrants implementation of antimicrobial stewardship for the prevention and containment of antimicrobial resistance in the study area and throughout the country.
- We recommend molecular technique for drug resistance detection, virulence profiling and genotyping in the country.
- Although the present study determined the genotypic characteristics of *S. aureus* clinical isolates for the first time in the country, we recommend a larger study representing the different regions of the country to be conducted for conclusive national data for policy makers, clinicians and researchers.

REFERENCES

Abdella M. 1996. Bacterial causes of bovine mastitis in Wondogenet, Ethiopia. *Zentralbl Veterinarmed B* 43(6):379-384.

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 Vet Res* 12(1):270.

Abera M, Habte T, Aragaw K, Asmare K, Sheferaw D. 2012. Major causes of mastitis and associated risk factors in smallholder dairy farms in and around Hawassa, Southern Ethiopia. *Trop Anim Health Prod* 44(6):1175-1179.

Al Jalaf M, Fadali H, Alanee R, Najjar F, Al Deesi Z, Seliem RM, Nilles EJ. 2018. Methicillin resistant Staphylococcus Aureus in emergency department patients in the United Arab Emirates. *BMC Emerg Med* 18(1):12.

Almaw G, Zerihun A, Asfaw Y. 2008. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. *Trop Anim Health Prod* 40(6):427-432.

Amenu D, Belachew T, Araya F. 2011. Surgical site infection rate and risk factors among obstetric cases of jimma university specialized hospital, southwest ethiopia. *Ethiop J Health Sci* 21(2):91-100.

Antoci E, Pinzone MR, Nunnari G, Stefani S, Cacopardo B. 2013. Prevalence and molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) among subjects working on bovine dairy farms. *Infez Med* 21(2):125-129.

Arcuri EF, Angelo FF, Guimaraes MF, Talon R, Borges Mde F, Leroy S, Loiseau G, Lange CC, Andrade NJ, Montet D. 2010. Toxigenic status of Staphylococcus aureus isolated from bovine raw milk and Minas frescal cheese in Brazil. *J Food Prot* 73(12):2225-2231.

Armand-Lefevre L, Ruimy R, Andremont A. 2005. Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis* 11(5):711-714.

Asadollahi P, Farahani NN, Mirzaii M, Khoramrooz SS, van Belkum A, Asadollahi K, Dadashi M, Darban-Sarokhalil D. 2018. Distribution of the Most Prevalent Spa Types among Clinical Isolates of Methicillin-Resistant and -Susceptible Staphylococcus aureus around the World: A Review. *Front Microbiol* 9:163.

Asai T, Hiki M, Baba K, Usui M, Ishihara K, Tamura Y. 2012. Presence of Staphylococcus aureus ST398 and ST9 in Swine in Japan. *Jpn J Infect Dis* 65(6):551-552.

Asiimwe BB, Baldan R, Trovato A, Cirillo DM. 2017a. Molecular epidemiology of Panton-Valentine Leukocidin-positive community-acquired methicillin resistant Staphylococcus aureus isolates in pastoral communities of rural south western Uganda. *BMC Infect Dis* 17(1).

Asiimwe BB, Baldan R, Trovato A, Cirillo DM. 2017b. 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 Infect Dis* 17(1):422.

Aslam N, Izhar M, Mehdi N. 2013. Frequency of methicillin-resistant Staphylococcus aureus nasal colonization among patients suffering from methicillin resistant Staphylococcus aureus bacteraemia. *Pak J Med Sci* 29(6):1430-1432.

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. *Pak J Biol Sci* 20(6):298-305.

Ayeni FA, Ruppitsch W, Allerberger F. 2018. Molecular characterization of clonal lineage and staphylococcal toxin genes from S. aureus in Southern Nigeria. *PeerJ* 6:e5204.

Ayepola OO, Olasupo NA, Egwari LO, Becker K, Schaumburg F. 2015. Molecular Characterization and Antimicrobial Susceptibility of Staphylococcus aureus Isolates from Clinical Infection and Asymptomatic Carriers in Southwest Nigeria. *PLoS One* 10(9):e0137531.

Azene MK, Beyene BA. 2011. Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia. *TJHR* 13(4):1-10.

Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, Soleimani M, Peerayeh SN. 2012. Genetic characterization of a vancomycin-resistant Staphylococcus aureus isolate from the respiratory tract of a patient in a university hospital in northeastern Iran. *J Clin Microbiol* 50(11):3581-3585.

Baranovich T, Zaraket H, Shabana, II, Nevzorova V, Turcutyuicov V, Suzuki H. 2010. Molecular characterization and susceptibility of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolates from hospitals and the community in Vladivostok, Russia. *Clin Microbiol Infect* 16(6):575-582.

Barber M. 1961. Methicillin-resistant staphylococci. *J Clin Pathol* 14:385-393.

Bassetti M, Carnelutti A, Righi E. 2017. The role of methicillin-resistant Staphylococcus aureus in skin and soft tissue infections. *Curr Opin Infect Dis* 30(2):150-157.

Becker K, Ballhausen B, Kock R, Kriegeskorte A. 2014. Methicillin resistance in Staphylococcus isolates: the "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. *Int J Med Microbiol* 304(7):794-804.

Becker K, Larsen AR, Skov RL, Paterson GK, Holmes MA, Sabat AJ, Friedrich AW, Kock R, Peters G, Kriegeskorte A. 2013. Evaluation of a modular multiplex-PCR methicillin-resistant Staphylococcus aureus detection assay adapted for mecC detection. *J Clin Microbiol* 51(6):1917-1919.

Ben Said M, Abbassi MS, Bianchini V, Sghaier S, Cremonesi P, Romano A, Gualdi V, Hassen A, Luini MV. 2016. Genetic characterization and antimicrobial resistance of Staphylococcus aureus isolated from bovine milk in Tunisia. *Lett Appl Microbiol* 63(6):473-481.

Benoit SR, Estivariz C, Mogdasy C, Pedreira W, Galiana A, Bagnulo H, Gorwitz R, Fosheim GE, McDougal LK, Jernigan D. 2008. Community strains of methicillin-resistant Staphylococcus aureus as potential cause of healthcare-associated infections, Uruguay, 2002-2004. *Emerg Infect Dis* 14(8):1216-1223.

Berrios-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, Reinke CE, Morgan S, Solomkin JS, Mazuski JE, Dellinger EP, Itani KMF, Berbari EF, Segreti J, Parvizi J, Blanchard J, Allen G, Kluytmans J, Donlan R, Schecter WP, Healthcare Infection Control Practices Advisory C. 2017. Centers for Disease Control and

Prevention Guideline for the Prevention of Surgical Site Infection, 2017. *JAMA Surg* 152(8):784-791.

Berube BJ, Bubeck Wardenburg J. 2013. Staphylococcus aureus alpha-toxin: nearly a century of intrigue. *Toxins (Basel)* 5(6):1140-1166.

Borgey F, Thibon P, Ertzscheid MA, Bernet C, Gautier C, Mourens C, Bettinger A, Aggoune M, Galy E, Lejeune B, Kadi Z. 2012. Pre-operative skin preparation practices: results of the 2007 French national assessment. *J Hosp Infect* 81(1):58-65.

Bouchiat C, Curtis S, Spiliopoulou I, Bes M, Cocuzza C, Codita I, Dupieux C, Giormezis N, Kearns A, Laurent F, Molinos S, Musumeci R, Prat C, Saadatian-Elahi M, Tacconelli E, Tristan A, Schulte B, Vandenesch F, Staphylococci ESGo, Staphylococcal I. 2017. MRSA infections among patients in the emergency department: a European multicentre study. *J Antimicrob Chemother* 72(2):372-375.

Brakstad OG, Aasbakk K, Maeland JA. 1992. Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. *J Clin Microbiol* 30(7):1654-1660.

Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, Wren MW. 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). *J Antimicrob Chemother* 56(6):1000-1018.

Bukowski M, Wladyka B, Dubin G. 2010. Exfoliative toxins of Staphylococcus aureus. *Toxins (Basel)* 2(5):1148-1165.

Casado-Verrier B, Gomez-Fernandez C, Pano-Pardo JR, Gomez-Gil R, Mingorance-Cruz J, Moreno-Alonso de Celada R, Herranz-Pinto P. 2012. [Community-acquired methicillin resistant Staphylococcus aureus skin and soft tissue infections in Madrid: prevalence study]. *Enferm Infecc Microbiol Clin* 30(6):300-306.

CDC. 2012. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Methicillin -Resistant Staphylococcus aureus, 2012. *Centers for Disease Control and Prevention, Atlanta, USA* Available via the Internet: http://www.cdc.gov/abcs/reports-findings/survreports/mrsa12.pdf.

CDC. 2014. Procedure-associated Module: Surgical Site Infection (SSI) Event. *Centers for Disease Control and Prevention, USA*:1-23.

Changchien CH, Chen SW, Chen YY, Chu C. 2016. Antibiotic susceptibility and genomic variations in Staphylococcus aureus associated with Skin and Soft Tissue Infection (SSTI) disease groups. *BMC Infect Dis* 16:276.

Chastre J, Blasi F, Masterton RG, Rello J, Torres A, Welte T. 2014. European perspective and update on the management of nosocomial pneumonia due to methicillin-resistant Staphylococcus aureus after more than 10 years of experience with linezolid. *Clin Microbiol Infect* 20 Suppl 4:19-36.

Cheung GY, Joo HS, Chatterjee SS, Otto M. 2014. Phenol-soluble modulins – critical determinants of staphylococcal virulence. *FEMS Microbiol Rev*:1-22.

Chiang HY, Herwaldt LA, Blevins AE, Cho E, Schweizer ML. 2014. Effectiveness of local vancomycin powder to decrease surgical site infections: a meta-analysis. *Spine J* 14(3):397-407.

Chuang YY, Huang YC. 2013. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. *Lancet Infect Dis* 13(8):698-708.

Cicconi-Hogan KM, Belomestnykh N, Gamroth M, Ruegg PL, Tikofsky L, Schukken YH. 2014. Short communication: Prevalence of methicillin resistance in coagulase-negative staphylococci and Staphylococcus aureus isolated from bulk milk on organic and conventional dairy farms in the United States. *J Dairy Sci* 97(5):2959-2964.

CLSI. 2014. M100-S24 - Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. *Clinical and Laboratory Standards Institute* 34(1):1-226.

Cole TS, Riordan A. 2013. Vancomycin dosing in children: what is the question? *Arch Dis Child* 98(12):994-997.

Cooper RA. 2013. Surgical site infections: epidemiology and microbiological aspects in trauma and orthopaedic surgery. *Int Wound J* 10 Suppl 1:3-8.

Costa FN, Belo NO, Costa EA, Andrade GI, Pereira LS, Carvalho IA, Santos RL. 2018. Frequency of enterotoxins, toxic shock syndrome toxin-1, and biofilm formation genes in Staphylococcus aureus isolates from cows with mastitis in the Northeast of Brazil. *Trop Anim Health Prod* 50(5):1089-1097.

CSA. 2017. Agricultural sample survey: Report on livestock and livestock characteristics. *Central Statistical Agency of Ethiopia* II:38-50.

Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. 2009. Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. *PLoS One* 4(8):e6800.

David MZ, Daum RS. 2010. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 23(3):616-687.

de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP, van Strijp JA. 2004. Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. *J Exp Med* 199(5):687-695.

De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW. 2012. Invited review: Mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. *J Dairy Sci* 95(3):1025-1040.

Dearborn AD, Dokland T. 2012. Mobilization of pathogenicity islands by Staphylococcus aureus strain Newman bacteriophages. *Bacteriophage* 2(2):70-78.

Deghorain M, Van Melderen L. 2012. The Staphylococci phages family: an overview. *Viruses* 4(12):3316-3335.

Dekker D, Wolters M, Mertens E, Boahen KG, Krumkamp R, Eibach D, Schwarz NG, Adu-Sarkodie Y, Rohde H, Christner M, Marks F, Sarpong N, May J. 2016. Antibiotic resistance and clonal diversity of invasive Staphylococcus aureus in the rural Ashanti Region, Ghana. *BMC Infect Dis* 16(1):720.

Deresinski S. 2013. The multiple paths to heteroresistance and intermediate resistance to vancomycin in Staphylococcus aureus. *J Infect Dis* 208(1):7-9.

Dessalegn L, Shimelis T, Tadesse E, Gebre-selassie S. 2014. Aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern: a hospital based cross-sectional study. *J Med Res* 3(2):018-023.

Deurenberg RH, Stobberingh EE. 2008. The evolution of Staphylococcus aureus. *Infect Genet Evol* 8(6):747-763.

Doyle ME, Hartmann FA, Lee Wong AC. 2012. Methicillin-resistant staphylococci: implications for our food supply? *Anim Health Res Rev* 13(2):157-180.

Dressler AE, Scheibel RP, Wardyn S, Harper AL, Hanson BM, Kroeger JS, Diekema DJ, Bender JB, Gray GC, Smith TC. 2012. Prevalence, antibiotic resistance and molecular characterisation of Staphylococcus aureus in pigs at agricultural fairs in the USA. *Vet Rec* 170(19):495.

Egyir B, Guardabassi L, Sorum M, Nielsen SS, Kolekang A, Frimpong E, Addo KK, Newman MJ, Larsen AR. 2014. Molecular epidemiology and antimicrobial susceptibility of clinical Staphylococcus aureus from healthcare institutions in Ghana. *PLoS One* 9(2):e89716.

Eiff CV, Becker K, Machka K, Stammer H, Peters G. 2001. Nasal carriage as a source of Staphylococcus aureus bacteremia. *N Engl J Med* 344(1):11-16

El-Gilany AH, Fathy H. 2009. Risk factors of recurrent furunculosis. *Dermatol Online J* 15(1):16.

El Shallaly GH, Hassan AN, Siddig NO, Mohammed RA, Osman EA, Ibrahim MA. 2012. Study of patients with community-acquired abscesses. *Surg Infect (Larchmt)* 13(4):250-256.

Elazhari M, Elhabchi D, Zerouali K, Dersi N, Elmalki A, Hassar M, Saile R, Timinouni M. 2011. Prevalence and distribution of superantigen toxin genes in clinical community isolates of Staphylococcus aureus. *J Bacteriol Parasitol* 2(1):1-6.

Elhaig MM, Selim A. 2014. Molecular and bacteriological investigation of subclinical mastitis caused by Staphylococcus aureus and Streptococcus agalactiae in domestic bovids from Ismailia, Egypt. *Trop Anim Health Prod*.

Ericsson Unnerstad H, Lindberg A, Persson Waller K, Ekman T, Artursson K, Nilsson-Ost M, Bengtsson B. 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. *Vet Microbiol* 137(1-2):90-97.

Erskine RJ. 2012. Vaccination strategies for mastitis. *Vet Clin North Am Food Anim Pract* 28(2):257-270.

Espinosa-Gongora C, Larsen J, Moodley A, Nielsen JP, Skov RL, Andreasen M, Guardabassi L. 2012. Farm-specific lineages of methicillin-resistant Staphylococcus aureus clonal complex 398 in Danish pig farms. *Epidemiol Infect* 140(10):1794-1799.

Fang HW, Chiang PH, Huang YC. 2014. Livestock-associated methicillin-resistant Staphylococcus aureus ST9 in pigs and related personnel in Taiwan. *PLoS One* 9(2):e88826.

Fitzgerald JR. 2012. Livestock-associated Staphylococcus aureus: origin, evolution and public health threat. *Trends Microbiol* 20(4):192-198.

Foster TJ, Geoghegan JA, Ganesh VK, Hook M. 2014. Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. *Nat Rev Microbiol* 12(1):49-62.

Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC. 2013. Isolation and characterization of methicillin-resistant

Staphylococcus aureus from pork farms and visiting veterinary students. *PLoS One* 8(1):e53738.

Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA. 2011. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis* 11(8):595-603.

Getahun K, Kelay B, Bekana M, Lobago F. 2008. Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Trop Anim Health Prod* 40(4):261-268.

Ghebremedhin B, Olugbosi MO, Raji AM, Layer F, Bakare RA, Konig B, Konig W. 2009. Emergence of a community-associated methicillin-resistant Staphylococcus aureus strain with a unique resistance profile in Southwest Nigeria. *J Clin Microbiol* 47(9):2975-2980.

Gonzalez DJ, Vuong L, Gonzalez IS, Keller N, McGrosso D, Hwang JH, Hung J, Zinkernagel A, Dixon JE, Dorrestein PC, Nizet V. 2014. Phenol Soluble Modulin (PSM) Variants of Community-Associated Methicillin-Resistant Staphylococcus aureus (MRSA) Captured Using Mass Spectrometry-Based Molecular Networking. *Mol Cell Proteomics* 13(5):1262-1272.

Gordon RJ, Lowy FD. 2008. Pathogenesis of methicillin-resistant Staphylococcus aureus infection. *Clin Infect Dis* 46 Suppl 5:S350-359.

Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. 2011. Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. *Int J Med Microbiol* 301(8):630-634.

Grumann D, Nubel U, Broker BM. 2014. Staphylococcus aureus toxins--their functions and genetics. *Infect Genet Evol* 21:583-592.

Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW. 2010. Geographic distribution of Staphylococcus aureus causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med* 7(1):e1000215.

Gulmez D, Sancak B, Ercis S, Karakaya J, Hascelik G. 2012. [Investigation of SCCmec types and Panton-Valentine leukocidin in community-acquired and nosocomial Staphylococcus aureus strains: comparing skin and soft tissue infections to the other infections]. *Mikrobiyol Bul* 46(3):341-351.

Haftu R, Taddele H, Gugsa G, Kalayou S. 2012. Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. *Trop Anim Health Prod* 44(7):1765-1771.

Hagos F, Alemseged F, Balcha F, Berhe S, Aregay A. 2014. Application of Nursing Process and Its Affecting Factors among Nurses Working in Mekelle Zone Hospitals, Northern Ethiopia. *Nurs Res Pract* 2014:675212.

Hakim ST, Arshed S, Iqbal M, Javaid S. 2007. Vancomycin sensitivity of Staphylococcus aureus isolates from hospital patients in Karachi, Pakistan. *Libyan J Med* 2(4):176-179.

He C, Xu S, Zhao H, Hu F, Xu X, Jin S, Yang H, Gong F, Liu Q. 2018. Leukotoxin and pyrogenic toxin Superantigen gene backgrounds in bloodstream and wound Staphylococcus aureus isolates from eastern region of China. *BMC Infect Dis* 18(1):395.

Hennekinne JA, De Buyser ML, Dragacci S. 2012. Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev* 36(4):815-836.

Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. 1997. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 40(1):135-136.

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. *Infect Chemother* 45(2):117-136.

Ibler KS, Kromann CB. 2014. Recurrent furunculosis - challenges and management: a review. *Clin Cosmet Investig Dermatol* 7:59-64.

Ito T, Hiramatsu K, Tomasz A, de Lencastre H, Perreten V, Holden MT, Coleman DC, Goering R, Giffard PM, Skov RL, Zhang K, Westh H, O'Brien F, Tenover FC, Oliveira DC, Boyle-Vavra S, Laurent F, Kearns AM, Kreiswirth B, Ko KS, Grundmann H, Sollid JE, John JF, Jr., Daum R, Soderquist B, Buist G. 2012. Guidelines for reporting novel mecA gene homologues. *Antimicrob Agents Chemother* 56(10):4997-4999.

Jaton L, Pillonel T, Jaton K, Dory E, Prod'hom G, Blanc DS, Tissot F, Bodenmann P, Greub G. 2016. Common skin infection due to Panton-Valentine leucocidin-producing Staphylococcus aureus strains in asylum seekers from Eritrea: a genome-based investigation of a suspected outbreak. *Clin Microbiol Infect* 22(8):739 e735-738.

Juhas M. 2013. Horizontal gene transfer in human pathogens. *Crit Rev Microbiol*.

Kahsay A, Mihret A, Abebe T, Andualem T. 2014. Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region, Ethiopia. *Arch Public Health* 72(1):16.

Katsande S, Matope G, Ndengu M, Pfukenyi DM. 2013. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort J Vet Res* 80(1):523.

Keefe G. 2012. Update on control of Staphylococcus aureus and Streptococcus agalactiae for management of mastitis. *Vet Clin North Am Food Anim Pract* 28(2):203-216.

Kejela T, Bacha K. 2013. Prevalence and antibiotic susceptibility pattern of methicillin-resistant Staphylococcus aureus (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. *Ann Clin Microbiol Antimicrob* 12:11.

Keynan Y, Rubinstein E. 2013. Staphylococcus aureus bacteremia, risk factors, complications, and management. *Crit Care Clin* 29(3):547-562.

Knight-Jones TJ, Hang'ombe MB, Songe MM, Sinkala Y, Grace D. 2016. Microbial Contamination and Hygiene of Fresh Cow's Milk Produced by Smallholders in Western Zambia. *Int J Environ Res Public Health* 13(5).

Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K. 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type

assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob Agents Chemother* 51(1):264-274.

Kotisso B, Aseffa A. 1998. Surgical wound infection in a teaching hospital in Ethiopia. *East Afr Med J* 75(7):402-405.

Krishna S, Miller LS. 2012. Host-pathogen interactions between the skin and Staphylococcus aureus. *Curr Opin Microbiol* 15(1):28-35.

Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K. 2001. Whole genome sequencing of meticillin-resistant Staphylococcus aureus. *Lancet* 357(9264):1225-1240.

Kwan T, Liu J, DuBow M, Gros P, Pelletier J. 2005. The complete genomes and proteomes of 27 Staphylococcus aureus bacteriophages. *Proc Natl Acad Sci U S A* 102(14):5174-5179.

Levine NS, Lindberg RB, Mason AD, Jr., Pruitt BA, Jr. 1976. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma* 16(2):89-94.

Li J, Echevarria KL, Traugott KA. 2017. beta-Lactam Therapy for Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Comparative Review of Cefazolin versus Antistaphylococcal Penicillins. *Pharmacotherapy* 37(3):346-360.

Lin J, Lin D, Xu P, Zhang T, Ou Q, Bai C, Yao Z. 2016. Non-hospital environment contamination with Staphylococcus aureus and methicillin-resistant Staphylococcus aureus: proportion meta-analysis and features of antibiotic resistance and molecular genetics. *Environ Res* 150:528-540.

Lin YC, Peterson ML. 2010. New insights into the prevention of staphylococcal infections and toxic shock syndrome. *Expert Rev Clin Pharmacol* 3(6):753-767.

Lindsay JA. 2014. Staphylococcus aureus genomics and the impact of horizontal gene transfer. *Int J Med Microbiol* 304(2):103-109.

Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, M JR, Talan DA, Chambers HF. 2011. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. *Clin Infect Dis* 52(3):e18-55.

Liu GY. 2009. Molecular pathogenesis of Staphylococcus aureus infection. *Pediatr Res* 65(5 Pt 2):71R-77R.

Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, Shirliff ME. 2016a. Staphylococcal chromosomal cassettes mec (SCCmec): A mobile genetic element in methicillin-resistant Staphylococcus aureus. *Microb Pathog* 101:56-67.

Liu Y, Xu Z, Yang Z, Sun J, Ma L. 2016b. Characterization of community-associated Staphylococcus aureus from skin and soft-tissue infections: a multicenter study in China. *Emerg Microbes Infect* 5(12):e127.

Locatelli C, Cremonesi P, Caprioli A, Carfora V, Ianzano A, Barberio A, Morandi S, Casula A, Castiglioni B, Bronzo V, Moroni P. 2017. Occurrence of methicillin-resistant

Staphylococcus aureus in dairy cattle herds, related swine farms, and humans in contact with herds. *J Dairy Sci* 100(1):608-619.

Loffler B, Hussain M, Grundmeier M, Bruck M, Holzinger D, Varga G, Roth J, Kahl BC, Proctor RA, Peters G. 2010. Staphylococcus aureus panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathog* 6(1):e1000715.

Lovseth A, Loncarevic S, Berdal KG. 2004. Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. *J Clin Microbiol* 42(8):3869-3872.

Macmorran E, Harch S, Athan E, Lane S, Tong S, Crawford L, Krishnaswamy S, Hewagama S. 2017. The rise of methicillin resistant Staphylococcus aureus: now the dominant cause of skin and soft tissue infection in Central Australia. *Epidemiol Infect* 145(13):2817-2826.

Magilner D, Byerly MM, Cline DM. 2008. The prevalence of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) in skin abscesses presenting to the pediatric emergency department. *N C Med J* 69(5):351-354.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18(3):268-281.

Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. 2013. Characterization of methicillin-resistant Staphylococcus aureus from skin and soft tissue infections in patients in Nairobi, Kenya. *Int J Infect Dis* 17(2):e115-119.

Malachowa N, DeLeo FR. 2010. Mobile genetic elements of Staphylococcus aureus. *Cell Mol Life Sci* 67(18):3057-3071.

Mama M, Abdissa A, Sewunet T. 2014. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia. *Ann Clin Microbiol Antimicrob* 13:14.

Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. 1999. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. *Am J Infect Control* 27(2):97-132; quiz 133-134; discussion 196.

Marasa BS, Revollo J, Iram S, Sung K, Xu J, Khan S. 2014. Draft Genome Sequence of a Methicillin-Resistant Staphylococcus aureus ST1413 Strain for Studying Genetic Mechanisms of Antibiotic Resistance. *Genome Announc* 2(2).

Mariem BJ, Ito T, Zhang M, Jin J, Li S, Ilhem BB, Adnan H, Han X, Hiramatsu K. 2013. Molecular characterization of methicillin-resistant Panton-valentine leukocidin positive staphylococcus aureus clones disseminating in Tunisian hospitals and in the community. *BMC Microbiol* 13:2.

Marimon JM, Villar M, Garcia-Arenzana JM, Caba Ide L, Perez-Trallero E. 2012. Molecular characterization of Staphylococcus aureus carrying the panton-valentine leucocidin genes in northern Spain. *J Infect* 64(1):47-53.

McCaig LF, McDonald LC, Mandal S, Jernigan DB. 2006. Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care. *Emerg Infect Dis* 12(11):1715-1723.

McCarthy AJ, Witney AA, Lindsay JA. 2012. Staphylococcus aureus temperate bacteriophage: carriage and horizontal gene transfer is lineage associated. *Front Cell Infect Microbiol* 2:6.

Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. 2012. Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA). *Curr Opin Microbiol* 15(5):588-595.

Mekonnen SA, Koop G, Melkie ST, Getahun CD, Hogeveen H, Lam T. 2017. Prevalence of subclinical mastitis and associated risk factors at cow and herd level in dairy farms in North-West Ethiopia. *Prev Vet Med* 145:23-31.

Mekonnen SA, Lam T, Hoekstra J, Rutten V, Tessema TS, Broens EM, Riesebos AE, Spaninks MP, Koop G. 2018. Characterization of Staphylococcus aureus isolated from milk samples of dairy cows in small holder farms of North-Western Ethiopia. *BMC Vet Res* 14(1):246.

Mekuria A, Asrat D, Woldeamanuel Y, Tefera G. 2013. Identification and antimicrobial susceptibility of *Staphylococcus aureus* isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. *Afr J Microbiol Res* 7(27):3501-3510.

Melaku S, Gebre-Selassie S, Damtie M, Alamrew K. 2012. Hospital acquired infections among surgical, gynaecology and obstetrics patients in Felege-Hiwot referral hospital, Bahir Dar, northwest Ethiopia. *Ethiop Med J* 50(2):135-144.

Mengesha RE, Kasa BG, Saravanan M, Berhe DF, Wasihun AG. 2014. Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. *BMC Res Notes* 7:575.

Missiakas DM, Schneewind O. 2013. Growth and laboratory maintenance of Staphylococcus aureus. *Curr Protoc Microbiol* Chapter 9:Unit 9C 1.

Mistry RD. 2013. Skin and soft tissue infections. *Pediatr Clin North Am* 60(5):1063-1082.

Mistry RD, Shapiro DJ, Goyal MK, Zaoutis TE, Gerber JS, Liu C, Hersh AL. 2014. Clinical management of skin and soft tissue infections in the U.S. Emergency Departments. *West J Emerg Med* 15(4):491-498.

Mohammed A, Seid ME, Gebrecherkos T, Tiruneh M, Moges F. 2017. Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Referral Hospital, Northwest Ethiopia. *Int J Microbiol* 2017:8953829.

Monaco M, Pedroni P, Sanchini A, Bonomini A, Indelicato A, Pantosti A. 2013. Livestock-associated methicillin-resistant Staphylococcus aureus responsible for human colonization and infection in an area of Italy with high density of pig farming. *BMC Infect Dis* 13:258.

Muileboom J, Hamilton M, Parent K, Makahnouk D, Kirlew M, Saginur R, Lam F, Kelly L. 2013. Community-associated methicillin-resistant Staphylococcus aureus in northwest Ontario: A five-year report of incidence and antibiotic resistance. *Can J Infect Dis Med Microbiol* 24(2):e42-44.

Mulu A, Moges F, Tessema B, Kassu A. 2006. Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital, Northwest Ethiopia. *Ethiop Med J* 44(2):125-131.

Mulu W, Kibru G, Beyene G, Damtie M. 2012. Postoperative Nosocomial Infections and Antimicrobial Resistance Pattern of Bacteria Isolates among Patients Admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. *Ethiop J Health Sci* 22(1):7-18.

Muluye D, Wondimeneh Y, Ferede G, Nega T, Adane K, Biadgo B, Tesfa H, Moges F. 2014. Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge at Gondar university hospital. *BMC Res Notes* 7:619.

Nam HM, Lee AL, Jung SC, Kim MN, Jang GC, Wee SH, Lim SK. 2011. Antimicrobial susceptibility of Staphylococcus aureus and characterization of methicillin-resistant Staphylococcus aureus isolated from bovine mastitis in Korea. *Foodborne Pathog Dis* 8(2):231-238.

Nichol KA, Adam HJ, Roscoe DL, Golding GR, Lagace-Wiens PR, Hoban DJ, Zhanel GG. 2013. Changing epidemiology of methicillin-resistant Staphylococcus aureus in Canada. *J Antimicrob Chemother* 68 Suppl 1:i47-55.

Nubel U, Strommenger B, Layer F, Witte W. 2011. From types to trees: reconstructing the spatial spread of Staphylococcus aureus based on DNA variation. *Int J Med Microbiol* 301(8):614-618.

O'Hara FP, Amrine-Madsen H, Mera RM, Brown ML, Close NM, Suaya JA, Acosta CJ. 2012. Molecular characterization of Staphylococcus aureus in the United States 2004-2008 reveals the rapid expansion of USA300 among inpatients and outpatients. *Microb Drug Resist* 18(6):555-561.

Ogston A. 1882. Micrococcus Poisoning. *J Anat Physiol* 17(Pt 1):24-58.

Ojulong J, Mwambu TP, Joloba M, Bwanga F, Kaddu-Mulindwa DH. 2009. Relative prevalence of methicilline resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. *Tanzan J Health Res* 11(3):149-153.

Oliveira D, Borges A, Simoes M. 2018. Staphylococcus aureus Toxins and Their Molecular Activity in Infectious Diseases. *Toxins (Basel)* 10(6).

Omuse G, Van Zyl KN, Hoek K, Abdulgader S, Kariuki S, Whitelaw A, Revathi G. 2016. Molecular characterization of Staphylococcus aureus isolates from various healthcare institutions in Nairobi, Kenya: a cross sectional study. *Ann Clin Microbiol Antimicrob* 15(1):51.

Oosthuysen WF, Orth H, Lombard CJ, Sinha B, Wasserman E. 2014. Population structure analyses of Staphylococcus aureus at Tygerberg Hospital, South Africa, reveals a diverse population, a high prevalence of Panton-Valentine leukocidin genes, and unique local methicillin-resistant S. aureus clones. *Clin Microbiol Infect* 20(7):652-659.

Ortega E, Abriouel H, Lucas R, Galvez A. 2010. Multiple roles of Staphylococcus aureus enterotoxins: pathogenicity, superantigenic activity, and correlation to antibiotic resistance. *Toxins (Basel)* 2(8):2117-2131.

Otto A, van Dijl JM, Hecker M, Becher D. 2014. The Staphylococcus aureus proteome. *Int J Med Microbiol* 304(2):110-120.

Otto M. 2012. MRSA virulence and spread. *Cell Microbiol* 14(10):1513-1521.

Otto M. 2013. Community-associated MRSA: what makes them special? *Int J Med Microbiol* 303(6-7):324-330.

Otto M. 2014. Staphylococcus aureus toxins. *Curr Opin Microbiol* 17:32-37.

Owens PL, Barrett ML, Raetzman S, Maggard-Gibbons M, Steiner CA. 2014. Surgical site infections following ambulatory surgery procedures. *JAMA* 311(7):709-716.

Panton PN, Came MB, Valentine FCO, Lond MRCP. 1932. Staphylococcal toxin. *Lancet* 219(5662):506–508.

Pardos de la Gandara M, Raygoza Garay JA, Mwangi M, Tobin JN, Tsang A, Khalida C, D'Orazio B, Kost RG, Leinberger-Jabari A, Coffran C, Evering TH, Coller BS, Balachandra S, Urban T, Parola C, Salvato S, Jenks N, Wu D, Burgess R, Chung M, de Lencastre H, Tomasz A. 2015. Molecular Types of Methicillin-Resistant Staphylococcus aureus and Methicillin-Sensitive S. aureus Strains Causing Skin and Soft Tissue Infections and Nasal Colonization, Identified in Community Health Centers in New York City. *J Clin Microbiol* 53(8):2648-2658.

Paterson GK, Harrison EM, Holmes MA. 2014. The emergence of mecC methicillin-resistant Staphylococcus aureus. *Trends Microbiol* 22(1):42-47.

Persson Y, Nyman AK, Gronlund-Andersson U. 2011. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet Scand* 53:36.

Peschel A, Otto M. 2013. Phenol-soluble modulins and staphylococcal infection. *Nat Rev Microbiol* 11(10):667-673.

Peton V, Le Loir Y. 2014. Staphylococcus aureus in veterinary medicine. *Infect Genet Evol* 21:602-615.

Plata K, Rosato AE, Wegrzyn G. 2009. Staphylococcus aureus as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. *Acta Biochim Pol* 56(4):597-612.

Porrero MC, Wassenaar TM, Gomez-Barrero S, Garcia M, Barcena C, Alvarez J, Saez-Llorente JL, Fernandez-Garayzabal JF, Moreno MA, Dominguez L. 2012. Detection of methicillin-resistant Staphylococcus aureus in Iberian pigs. *Lett Appl Microbiol* 54(4):280-285.

Portillo BC, Moreno JE, Yomayusa N, Alvarez CA, Cardozo BE, Perez JA, Diaz PL, Ibanez M, Mendez-Alvarez S, Leal AL, Gomez NV. 2013. Molecular epidemiology and characterization of virulence genes of community-acquired and hospital-acquired methicillin-resistant Staphylococcus aureus isolates in Colombia. *Int J Infect Dis* 17(9):e744-749.

Postma B, Poppelier MJ, van Galen JC, Prossnitz ER, van Strijp JA, de Haas CJ, van Kessel KP. 2004. Chemotaxis inhibitory protein of Staphylococcus aureus binds specifically to the C5a and formylated peptide receptor. *J Immunol* 172(11):6994-7001.

Price JR, Didelot X, Crook DW, Llewelyn MJ, Paul J. 2013. Whole genome sequencing in the prevention and control of Staphylococcus aureus infection. *J Hosp Infect* 83(1):14-21.

Pulido MR, Garcia-Quintanilla M, Martin-Pena R, Cisneros JM, McConnell MJ. 2013. Progress on the development of rapid methods for antimicrobial susceptibility testing. *J Antimicrob Chemother* 68(12):2710-2717.

Ram G, Chen J, Kumar K, Ross HF, Ubeda C, Damle PK, Lane KD, Penades JR, Christie GE, Novick RP. 2012. Staphylococcal pathogenicity island interference with helper phage reproduction is a paradigm of molecular parasitism. *Proc Natl Acad Sci U S A* 109(40):16300-16305.

Ravishankar A, Singh S, Rai S, Sharma N, Gupta S, Thawani R. 2014. Socio-economic profile of patients with community-acquired skin and soft tissue infections in Delhi. *Pathog Glob Health* 108(6):279-282.

Riva A, Borghi E, Cirasola D, Colmegna S, Borgo F, Amato E, Pontello MM, Morace G. 2015. Methicillin-Resistant Staphylococcus aureus in Raw Milk: Prevalence, SCCmec Typing, Enterotoxin Characterization, and Antimicrobial Resistance Patterns. *J Food Prot* 78(6):1142-1146.

Rodriguez M, Hogan PG, Burnham CA, Fritz SA. 2014. Molecular epidemiology of Staphylococcus aureus in households of children with community-associated S aureus skin and soft tissue infections. *J Pediatr* 164(1):105-111.

Saeed K, Gould I, Esposito S, Ahmad-Saeed N, Ahmed SS, Alp E, Bal AM, Bassetti M, Bonnet E, Chan M, Coombs G, Dancer SJ, David MZ, De Simone G, Dryden M, Guardabassi L, Hanitsch LG, Hijazi K, Kruger R, Lee A, Leistner R, Pagliano P, Righi E, Schneider-Burrus S, Skov RL, Tattevin P, Van Wamel W, Vos MC, Voss A, International Society of C. 2018. Panton-Valentine leukocidin-positive Staphylococcus aureus: a position statement from the International Society of Chemotherapy. *Int J Antimicrob Agents* 51(1):16-25.

Santosaningsih D, Santoso S, Setijowati N, Rasyid HA, Budayanti NS, Suata K, Widhyatmoko DB, Purwono PB, Kuntaman K, Damayanti D, Prakoeswa CRS, Laurens M, van Nierop JWI, Nanninga GL, Oudenes N, de Regt M, Snijders SV, Verbrugh HA, Severin JA. 2018. Prevalence and characterisation of Staphylococcus aureus causing community-acquired skin and soft tissue infections on Java and Bali, Indonesia. *Trop Med Int Health* 23(1):34-44.

Santy-Tomlinson J. 2018. Antimicrobial resistance stewardship: it's up to us all. *Int J Orthop Trauma Nurs* 28:1-3.

Sartori C, Boss R, Bodmer M, Leuenberger A, Ivanovic I, Graber HU. 2018. Sanitation of Staphylococcus aureus genotype B-positive dairy herds: A field study. *J Dairy Sci* 101(8):6897-6914.

Savage JW, Anderson PA. 2013. An update on modifiable factors to reduce the risk of surgical site infections. *Spine J* 13(9):1017-1029.

Schelin J, Susilo YB, Johler S. 2017. Expression of Staphylococcal Enterotoxins under Stress Encountered during Food Production and Preservation. *Toxins (Basel)* 9(12).

Schmidt T, Kock MM, Ehlers MM. 2015. Diversity and antimicrobial susceptibility profiling of staphylococci isolated from bovine mastitis cases and close human contacts. *J Dairy Sci* 98(9):6256-6269.

Schmidt T, Kock MM, Ehlers MM. 2017. Molecular Characterization of Staphylococcus aureus Isolated from Bovine Mastitis and Close Human Contacts in South African Dairy Herds: Genetic Diversity and Inter-Species Host Transmission. *Front Microbiol* 8:511.

Seni J, Bwanga F, Najjuka CF, Makobore P, Okee M, Mshana SE, Kidenya BR, Joloba ML, Kateete DP. 2013a. Molecular characterization of Staphylococcus aureus from patients with surgical site infections at Mulago Hospital in Kampala, Uganda. *PLoS One* 8(6):e66153.

Seni J, Najjuka CF, Kateete DP, Makobore P, Joloba ML, Kajumbula H, Kapesa A, Bwanga F. 2013b. Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Uganda. *BMC Res Notes* 6:298.

Seyoum B, Kefyalew H, Abera B, Abdela N. 2018. Prevalence, risk factors and antimicrobial susceptibility test of Staphylococcus aureus in Bovine cross breed mastitic milk in and around Asella town, Oromia regional state, southern Ethiopia. *Acta Trop* 177:32-36.

Seyoum ET, Mekonene TK, Woldetsadik DA, Zewudie BM, Gebreyes WA. 2016. Enterotoxin gene profile of Staphylococcus aureus isolates recovered from bovine milk produced in central Ethiopia. *J Infect Dev Ctries* 10(2):138-142.

Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. 2013. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. *Lancet Infect Dis* 13(1):43-54.

Shearer JE, Wireman J, Hostetler J, Forberger H, Borman J, Gill J, Sanchez S, Mankin A, Lamarre J, Lindsay JA, Bayles K, Nicholson A, O'Brien F, Jensen SO, Firth N, Skurray RA, Summers AO. 2011. Major families of multiresistant plasmids from geographically and epidemiologically diverse staphylococci. *G3 (Bethesda)* 1(7):581-591.

Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. *J Clin Microbiol* 37(11):3556-3563.

Shore AC, Coleman DC. 2013. Staphylococcal cassette chromosome mec: recent advances and new insights. *Int J Med Microbiol* 303(6-7):350-359.

Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. 2008. Vancomycin-resistant Staphylococcus aureus in the United States, 2002-2006. *Clin Infect Dis* 46(5):668-674.

Silva NC, Guimaraes FF, Manzi MP, Budri PE, Gomez-Sanz E, Benito D, Langoni H, Rall VL, Torres C. 2013. Molecular characterization and clonal diversity of methicillin-susceptible Staphylococcus aureus in milk of cows with mastitis in Brazil. *J Dairy Sci* 96(11):6856-6862.

Silva NC, Guimaraes FF, Manzi MP, Junior AF, Gomez-Sanz E, Gomez P, Langoni H, Rall VL, Torres C. 2014. Methicillin-resistant Staphylococcus aureus of lineage ST398 as cause of mastitis in cows. *Lett Appl Microbiol* 59(6):665-669.

Sina H, Ahoyo TA, Moussaoui W, Keller D, Bankole HS, Barogui Y, Stienstra Y, Kotchoni SO, Prevost G, Baba-Moussa L. 2013. Variability of antibiotic susceptibility and toxin production of Staphylococcus aureus strains isolated from skin, soft tissue, and bone related infections. *BMC Microbiol* 13:188.

Skinner D, Keefer CS. 1941. Significance of bacteremia caused by Staphylococcus aureus: a study of one hundred and twenty-two cases and a review of the literature concerned with experimental infection in animals. *Arch Intern Med* 68(5):851-875.

Smith EM, Needs PF, Manley G, Green LE. 2014. Global distribution and diversity of ovine-associated Staphylococcus aureus. *Infect Genet Evol* 22:208-215.

Smith TC. 2015. Livestock-associated Staphylococcus aureus: the United States experience. *PLoS Pathog* 11(2):e1004564.

Spaan AN, Henry T, van Rooijen WJ, Perret M, Badiou C, Aerts PC, Kemmink J, de Haas CJ, van Kessel KP, Vandenesch F, Lina G, van Strijp JA. 2013. The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. *Cell Host Microbe* 13(5):584-594.

Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, Aarestrup FM, Kearns AM, Larsen AR, Skov RL, Fitzgerald JR. 2013. Livestock origin for a human pandemic clone of community-associated methicillin-resistant Staphylococcus aureus. *MBio* 4(4).

Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, Mackenzie FM. 2012. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. *Int J Antimicrob Agents* 39(4):273-282.

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. 2012. Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). *Clin Microbiol Infect* 18(4):395-400.

Stenstrom R, Grafstein E, Romney M, Fahimi J, Harris D, Hunte G, Innes G, Christenson J. 2009. Prevalence of and risk factors for methicillin-resistant Staphylococcus aureus skin and soft tissue infection in a Canadian emergency department. *CJEM* 11(5):430-438.

Tang YW, Stratton CW. 2010. Staphylococcus aureus: An old pathogen with new weapons. *Clin Lab Med* 30(1):179-208.

Tarekgne EK, Skjerdal T, Skeie S, Rudi K, Porcellato D, Felix B, Narvhus JA. 2016. Enterotoxin Gene Profile and Molecular Characterization of Staphylococcus aureus Isolates from Bovine Bulk Milk and Milk Products of Tigray Region, Northern Ethiopia. *J Food Prot* 79(8):1387-1395.

Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33(9):2233-2239.

Tesfahunegn Z, Asrat D, Woldeamanuel Y, Estifanos K. 2009. Bacteriology of surgical site and catheter related urinary tract infections among patients admitted in Mekelle Hospital, Mekelle, Tigray, Ethiopia. *Ethiop Med J* 47(2):117-127.

Thati V, Shivannavar CT, Gaddad SM. 2011. Vancomycin resistance among methicillin resistant Staphylococcus aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. *Indian J Med Res* 134(5):704-708.

Tigabu E, Kassa T, Asrat D, Alemayehu H, Sinmegn T, Adkins PRF, Gebreyes W. 2015. Phenotypic and genotypic characterization of *Staphylococcus aureus* isolates recovered from bovine milk in central highlands of Ethiopia. *Afr J Microbiol Res* 9(44):2209-2217.

Truong H, Shah SS, Ludmir J, Twananana EO, Bafana M, Wood SM, Moffat H, Steenhoff AP. 2011. Staphylococcus aureus skin and soft tissue infections at a tertiary hospital in Botswana. *S Afr Med J* 101(6):413-416.

Turkyilmaz S, Tekbiyik S, Oryasin E, Bozdogan B. 2010. Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant Staphylococcus aureus isolated from bovine milk. *Zoonoses Public Health* 57(3):197-203.

Turlej A, Hryniewicz W, Empel J. 2011. Staphylococcal cassette chromosome mec (Sccmec) classification and typing methods: an overview. *Pol J Microbiol* 60(2):95-103.

Udo EE. 2013. Community-acquired methicillin-resistant Staphylococcus aureus: the new face of an old foe? *Med Princ Pract* 22 Suppl 1:20-29.

Udo EE, Pearman JW, Grubb WB. 1993. Genetic analysis of community isolates of methicillin-resistant Staphylococcus aureus in Western Australia. *J Hosp Infect* 25(2):97-108.

van Belkum A, Dunne WM, Jr. 2013. Next-generation antimicrobial susceptibility testing. *J Clin Microbiol* 51(7):2018-2024.

van der Meeren BT, Millard PS, Scacchetti M, Hermans MH, Hilbink M, Concelho TB, Ferro JJ, Wever PC. 2014. Emergence of methicillin resistance and Panton-Valentine leukocidin positivity in hospital- and community-acquired Staphylococcus aureus infections in Beira, Mozambique. *Trop Med Int Health* 19(2):169-176.

van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J. 2007. Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans. *Emerg Infect Dis* 13(12):1834-1839.

van Rijen MM, Van Keulen PH, Kluytmans JA. 2008. Increase in a Dutch hospital of methicillin-resistant Staphylococcus aureus related to animal farming. *Clin Infect Dis* 46(2):261-263.

Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J. 2003. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 9(8):978-984.

Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, Butaye P. 2010. Methicillin-resistant Staphylococcus aureus (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Vet Microbiol* 144(1-2):166-171.

Vanegas Munera JM, Ocampo Rios AM, Urrego DM, Jimenez Quiceno JN. 2017. In vitro susceptibility of methicillin-resistant Staphylococcus aureus isolates from skin and soft tissue infections to vancomycin, daptomycin, linezolid and tedizolid. *Braz J Infect Dis* 21(5):493-499.

Varatanović N, Podžo M, Mutevelić T, Podžo K, Čengić B, Hodžić A, Hodžić E. 2010. Use of California mastitis test, somatic cells count and bacteriological findings in diagnostics of subclinical mastitis. *Biotechnol Anim Husb* 26:65-74.

Verkade E, Kluytmans J. 2014. Livestock-associated Staphylococcus aureus CC398: animal reservoirs and human infections. *Infect Genet Evol* 21:523-530.

Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. 2005. Methicillin-resistant Staphylococcus aureus in pig farming. *Emerg Infect Dis* 11(12):1965-1966.

Vourli S, Vagiakou H, Ganteris G, Orfanidou M, Polemis M, Vatopoulos A, Malamou-Ladas H. 2009. High rates of community-acquired, Panton-Valentine leukocidin (PVL)- positive methicillin-resistant S. aureus (MRSA) infections in adult outpatients in Greece. *Euro Surveill* 14(2).

Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, Mevius DJ, Bosch T, Van Duijkeren E. 2009. Unexpected sequence types in livestock associated methicillin-resistant Staphylococcus aureus (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. *Vet Microbiol* 139(3-4):405-409.

Waness A. 2010. Revisiting Methicillin-Resistant Staphylococcus aureus Infections. *J Glob Infect Dis* 2(1):49-56.

Wang M, Zheng Y, Mediavilla JR, Chen L, Kreiswirth BN, Song Y, Yang R, Du H. 2017. Hospital Dissemination of tst-1-Positive Clonal Complex 5 (CC5) Methicillin-Resistant Staphylococcus aureus. *Front Cell Infect Microbiol* 7:101.

Wang SH, Hines L, van Balen J, Mediavilla JR, Pan X, Hoet AE, Kreiswirth BN, Pancholi P, Stevenson KB. 2015. Molecular and clinical characteristics of hospital and

community onset methicillin-resistant Staphylococcus aureus strains associated with bloodstream infections. *J Clin Microbiol* 53(5):1599-1608.

Watanabe S, Ohnishi T, Yuasa A, Kiyota H, Iwata S, Kaku M, Watanabe A, Sato J, Hanaki H, Manabe M, Suzuki T, Otsuka F, Aihara M, Iozumi K, Tamaki T, Funada Y, Shinozaki M, Kobayashi M, Okuda M, Kikyo G, Kikuchi K, Okada Y, Takeshima M, Kaneko O, Ogawa N, Ito R, Okuyama R, Shimada S, Shimizu T, Hatta N, Manabu M, Tsutsui K, Tanaka T, Miyachi Y, Asada H, Furukawa F, Kurokawa I, Iwatsuki K, Hide M, Muto M, Yamamoto O, Niihara H, Takagaki K, Kubota Y, Sayama K, Sano S, Furue M, Kanekura T. 2017. The first nationwide surveillance of antibacterial susceptibility patterns of pathogens isolated from skin and soft-tissue infections in dermatology departments in Japan. *J Infect Chemother* 23(8):503-511.

Wendlandt S, Fessler AT, Monecke S, Ehricht R, Schwarz S, Kadlec K. 2013. The diversity of antimicrobial resistance genes among staphylococci of animal origin. *Int J Med Microbiol* 303(6-7):338-349.

WHO. 2017. WHO guidelines on use of medically important antimicrobials in food-producing animals. Geneva: World Health Organization.

Worthing KA, Abraham S, Pang S, Coombs GW, Saputra S, Jordan D, Wong HS, Abraham RJ, Trott DJ, Norris JM. 2018. Molecular Characterization of Methicillin-Resistant Staphylococcus aureus Isolated from Australian Animals and Veterinarians. *Microb Drug Resist* 24(2):203-212.

Wu D, Wang Q, Yang Y, Geng W, Wang Q, Yu S, Yao K, Yuan L, Shen X. 2010. Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible Staphylococcus aureus from skin/soft tissue infections in a children's hospital in Beijing, China. *Diagn Microbiol Infect Dis* 67(1):1-8.

Xu SX, McCormick JK. 2012. Staphylococcal superantigens in colonization and disease. *Front Cell Infect Microbiol* 2:52.

Yamamoto LG. 2017. Treatment of Skin and Soft Tissue Infections. *Pediatr Emerg Care* 33(1):49-55.

Yao D, Yu FY, Qin ZQ, Chen C, He SS, Chen ZQ, Zhang XQ, Wang LX. 2010. Molecular characterization of Staphylococcus aureus isolates causing skin and soft tissue infections (SSTIs). *BMC Infect Dis* 10:133.

Yoong P, Torres VJ. 2013. The effects of Staphylococcus aureus leukotoxins on the host: cell lysis and beyond. *Curr Opin Microbiol* 16(1):63-69.

Zadoks RN, Middleton JR, McDougall S, Katholm J, Schukken YH. 2011. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *J Mammary Gland Biol Neoplasia* 16(4):357-372.

Zarei Koosha R, Mahmoodzadeh Hosseini H, Mehdizadeh Aghdam E, Ghorbani Tajandareh S, Imani Fooladi AA. 2016. Distribution of tsst-1 and mecA Genes in Staphylococcus aureus Isolated From Clinical Specimens. *Jundishapur J Microbiol* 9(3):e29057.

Zecconi A, Scali F. 2013. Staphylococcus aureus virulence factors in evasion from innate immune defenses in human and animal diseases. *Immunol Lett* 150(1-2):12-22.

Zenebe Y, Tibebu M, Tulu B, Mekonnen D, Mekonnen Z. 2018. Methicillin-resistant Staphylococcus aureus with genotyping method among human immunodeficiency virus positive pediatric patients in Northwest Ethiopia: A cross-sectional study design. *Ethiop J Health Dev* 32(3):1-8.

ANNEXES

Annex I: Information sheets

Annex I-A: Information sheet for Adult study participants with skin and soft tissue infections

I-A: English Version

Title of the project: Phenotypic and molecular characterization of *Staphylococcus aureus* from human and animals in Mekele, Northern Ethiopia

Principal investigator: Alem Abrha (BSc, MSc, PhD candidate in Medical Microbiology)

Organization: Addis Ababa University; college of Health Sciences; Department of Microbiology, Immunology and Parasitology

Sponsors: Addis Ababa University**,** the Ohio State University

Introduction: *S. aureus* is a commensal and dangerous pathogen capable of infecting virtually every tissue of the body. Skin and soft tissue infections in the community, and surgical site infections in the health care setting are mainly caused by this bacterium. Besides to human infections, *S. aureus* can also colonize and infect animals; where mastitis in dairy animals being the best example. Studies from abroad demonstrated that infected animals by *S. aureus* can act as a reservoir for zoonotic infections of humans. Despite its importance to public and animal health, data regarding molecular types, zoonotic transmission and toxin characteristics of *S. aureus* are lacking in Ethiopia.

Study participants: This study involves patients with community onset skin and soft tissue infections, postoperative wound infections at Ayder referral Hospital; dairy cows and farm workers in and around Mekele. Therefore, you are kindly invited to participate in this study.

Purpose of the study: to determine the burden and drug resistance pattern of *Staphylococcus aureus* from community onset skin and soft tissue infections, post-operative wound infections, bovine mastitis and dairy farm workers; to understand the molecular types of *S. aureus* isolates and their virulence characteristics in the study setting.

Duration: the duration of this study depends on the availability of study participants; but it is estimated that it can take about four months. However, laboratory sample from you will be collected only once.

Procedure: the procedure is easy and simple; first you will be asked few questions and then wound swab/ pus sample will be collected by trained nurse. The sample will be transported to laboratory for analysis. If *S. aureus* is isolated, it will be transported to abroad for further investigation.

Risk and discomfort: You may feel minor discomfort during sample collection. However, to minimize discomfort; collection will be performed by trained nurse following appropriate precaution.

Expected benefits: The laboratory investigations will be performed free of charge and results will be communicated with your physician; so that, you can get appropriate medical care. Besides the direct benefit you can get, the information gained from you and others will help to consider *S. aureus* infection prevention and control strategy at local or national level.

Confidentiality: We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. If a research article or publication comes from this study, you will not be identified by name. The information we collect as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

Voluntary Participation and Withdrawal: The participation is completely voluntary and you have the right not to participate in this study. You can stop yourself from participating in the study at any time after giving your consent. This decision will not affect in any way your current or future medical care in the health facility.

Contact information: If you have any questions about this study you can ask now or contact the following investigators and the ethics committee for further information. Alem Abrha (Tel 0911784037); Dr. Daniel Asrat (Tel 0911223019); Dr. Yimtubezenash Woldeamanuel (Tel 0911225832); IRB-CHS (Tel : +251-118961396).

I-A: Information sheet: Amharic Version

የጥናቱ ርእስ፡ *'ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ አኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ'*
የዋና ተመራማሪ ሥም፡ አለም አብርሃ (በሜዲካል ማይክሮባዮሎጂ የሦስተኛ ዲግሪ ተማሪ)
የድርጅትሥም፡ አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ የማይክሮባዮሎጂ ኢሙኖሎጂ እና ፓራሲቶሎጂ ት/ት ክፍል
ዋና ስፖንሰር፡ አዲስ አበባ ዩኒቨርሲቲ ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ

መግብያ፡ ስታፊሎኮከስ ኦውሪየስ በተለያዩ የሰዉንታችን ክፍሎች የሚገኝና በየትኛዉም የሰዉነታችን ክፍል በሽታ ማስከተል የሚችል ባክተርያ ነው፡፡ ስታፊሎኮከስ ኦውሪየስ የቆዳ እና የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን በማምጣት ግንባር ቀደም ነው፡፡ ይህ ባክተርያ ከሰው አልፎ በተለያዩ እንስሳት የተለያዩ የበሽታ ዓይነቶችን በማምጣት የሚታወቅ ሲሆን፤ ከነዚህ መካከል በወተት ላሞች የጡት ኢነፍክሽን (ማስታይተስ) ዋነኛው ነው፡፡ በባክተርያው የተጠቁት እንስሳት ባክተርያዉን ወደ ሰው ማስተላለፍ እንደሚችሉ ከአገራችን ዉጭ የተሰሩ ጥናቶች ያመላክታሉ፡፡ ስታፊሎኮከስ ኦውሪየስ በሰውና እንስሳት የሚያመጣው የጤና ችግር ከፍተኛ ቢሆንም በአገራችን በዚህ ባክተርያ ዙርያ ያለው በምርምር የተደገፈ መረጃ ውስን ነው፡፡ በተለይ በአገራችን ያሉትን የስታፊሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸው፣ ባከተርያው በሰውና እንስሳት መሃከል ሰለመተላለፉ እና ባክተርያው የያዛቸው መርዛማ ነገሮች (ቶክሲንስ) ምንም ዓይነት መረጃ የለም፡፡

የጥናቱ ተሳታፊዎች፡ ይህ ጥናት በህብረተሰብ ዉስጥ የተከሰተ የቆዳ ኢንፌክሽን ያላቸው፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን ያላቸው ፤ እንዲሁም በወተት እርሻ ዉስጥ የሚገኙ ሰራተኞችና ላሞች ያካትታል፡፡ በመሆኑም በዚህ ጥናት እንዲሳተፉ/ልጅዎ እነዲሳተፍ እንዲፈቅዱ በአክብሮት ተጋብዘዋል፡፡

የጥናቱ ዓላማ፡ በስታፊሎኮከስ ኦውሪየስ የሚመጣዉን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የላሞች የጡት እንፌክሽን ለማወቅ፤ እነዚህን በሽታዎችን ያመጡ የስታፊሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸዉን ለመረዳት እና ባክተርያው በሰውና እንስሳት መሃከል ያለዉን የመተላለፍ ሁኔታ ለማወቅ ነው፡፡

ጥናቱ የሚወስደው ጊዜ፡ ይህ ጥናት በቆዳ ኢንፌክሽን ወደ ሆስፒታሎቹ በሚመጡ ህምመተኞች ብዛት የሚወሰን ቢሆንም እሰከ አራት ወራት ይወስዳል ተብሎ ይገመታል፡፡ ይሁን እንጂ ከእርስዎ የሚወሰደው የላቦራቶሪ ናሙና አንዴ ቢቻ ነው፡፡

በጥናቱ የሚከናወኑ ተግባራት በቅደም ተከተል፡ በቅድምያ ጥቂት ጥያቄዎች ይጠየቃሉ፡፡ በመቀጠልም ኢንፌክሽኑ ካለበት ቦታየላቦራቶሪ ናሙና በሰለጠነ/ች ነርስ ይወሰዳል፡፡ ናሙናው ለምርመራ ወደ ላቦራቶሪ ይሄድና ስታፊሎኮከስ ኦውሪየስ ከተገኘ ለተጨማሪ ምርመራ ወደ ዉጭ ሃገር ይሄዳል፡፡

በጥናቱ ሲሳተፉ ሊፈጠር የሚችል ችግር፡ የቆዳ ኢንፌክሽን ካለበት ቦታ ናሙና በሚወሰድበት ጊዜ በጣም አነስተኛ ህመም ሊኖር ይችላል፡፡ ነገር ግን ይህንን ለመቀነስ ናሙናዉን በሰለጠነ/ች ነርስ በጥንቃቄ ይወሰዳል፡፡

የጥናቱ ጠቀሜታ፡ የላቦራቶሪ ምርመራው በነፃ የሚደረግ ሲሆን ዉጤቱን ለተሻለ ህክምና ይረዳዎ ዘንድ ለሚከታተልዎት ሀኪም ይሰጣል፡፡ በዚህ ጥናት በመሳተፍዎ ለእርስዎ ከሚሰጠው ቀጥተኛ ጥቅም በላይ ከእርስዎ እና ከሌሎች የሚገኘው መረጃ በአገራችን በስታፊሎኮከስ ኦውሪየስ አማካኝነት የሚከሰተዉን የቆዳ ኢንፌክሽን የመከላከያና የቁጥጥር ዘዴ ለመንደፍ ትልቅ ጠቀሜታ ይኖረዋል፡፡

ሚስጢራዊነት፡ የእርስዎ ክብርና ሚሰጢር በአስተማማኝ ሁኔታ እንጠብቃለን፡፡ እርስዎ የሚለይ መረጃ ከጥናት ቡደኑ ዉጭ ለማንም አናካፍልም፡፡ የጥናቱ ዉጤት ሲታተም የእርስዎ ሥም አይፃፍም፡፡ የምንሰበስበው መረጃ ቁልፍ ባለው ሳጥን የሚቀመጥ ሲሆን በኮመፒውተር የሚቀመጠዉን መረጃ ደገሞ በፓስዎረድ ይቆለፋል፡፡

የተሳትፎ ፈቃደኝነት፡ በዚህ ጥናት መሳተፍ ሙሉበሙሉ በፈቃደኝት የተመሰረተ ሲሆን ያለመሳተፍ መብትዎ የተጠበቀ ነው፡፡ ፈቃደኝነትዎ ከሰጡ ቡኃላም ቢሆን በማነኛዉም ሰዓት ከጥናቱ ራስዎን ማግለል ይችላሉ፡፡ እነዲህ በመወሰንዎ በአሁኑ ይሁን የወደፊት ህክምናዎ አገልግሎት በዚህ ጤና ተቋም የሚፈጥርብዎ ተፅዕኖ የለም፡፡

አድራሻ፡ በጥናቱ ዙርያ ማነኛዉም ዓይነት ጥያቄ አሁን መጠየቅ የሚችሉ ሲሆን ተጨማሪ ማብራርያ ቢፈልጉ ለሚከተሉት ተመራማሪዎችና የሥነምግባር ኮሚቴ በስልክ ማናገር ይችላሉ፡፡ አለም አብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡0911225832)፣ የአዲስ አበባ ዩኒቨርሲቲ የምርምር ስነምግባር ኮሚቴ (ስልክ፡ 0118961396)

I-A: Information sheet: TigrignaVersion

ትግርኛ ቅዳሕ

አርእስቲ ምርምር ፅንዓት፡ *'ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ አኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ'*

ሽም ዋና ተመራማሪ፡ *ኣለም ኣብርሃ (ብሜዲካል ማይክሮባዮሎጂ ተምሃራይ ሳልሳይ ዲግሪ)*

ሽም ድርጅት፡ *ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኮሌጅ ጥዕና ሳይንስ ፣ ክፍሊ ት/ቲ ማይክሮባዮሎጂ፣ ኢሙኖሎጂን ፓራሲቶሎጂን*

ዋና ስፖንሰር፡ *ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ*

መእተዊ፡ *ስታፊሎኮከስ ኦውሪየስ ኣብ ዝተፈላለዩ ኣካላት ሰዉነትና ዝርከብን ኣብ ኩሉ ክፍሊ ኣካላት ሰዉነትና ኢንፌክሽን ከምፅእ ዝኽእል ባክተርያ እዩ፡፡ ስታፊሎኮከስ ኦውሪየስ ኢንፌክሽን ቆርበትን ድሕሪ-መጥባሕቲ ቑስልን ብምምፃእ ቀዳማይ እዩ፡፡ እዚ ባክተርያ ካብ ሰብ ወፃኢ ኣብ ዝተፈላለዩ እንስሳታት ኢንፌክሽን ዘምፅእ እንትኾን፤ ኣብ ናይ ፀባ ከፍቲ ኢነፍክሽን ጡብ (ማስታይተስ) ብምምፃእ ይፍለጥ፡፡ ብስታፊሎኮከስ ኦውሪየስ ዝተጠቕዑ እንስሳት እቲ ባክተርያ ናብ ሰብ ምምሕልላፍ ከምዝኽእል ካብ ሃገርና ወፃኢ ዝተሰርሑ ምርምር ፅንዓታት የረድኡ፡፡ ስታፊሎኮከስ ኦውሪየስ ኣብ ሰብን እንስሳታትን ዘምፅኦ ሳዕቤን ጥዕና ዓብዪኳ እንተኾነ ኣብ ሃገርና ኣብዚ ባክተርያ ዘሎ ብምርምር ዝተደገፈ ሓበሬታ ውስን እዩ፡፡ ብፍላይ ኣብ ሃገርና ዘለዉ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ስርጭቶምን፣ እዚ ባክተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት፣ እዚ ባክተርያ ዝተሸከሞም ኩነታት መርዛማ ነገራት (ቶክሲንስ) ምንም ዓይነት መረዳእታ የለን፡፡*

ተሳተፍቲ ምርምር ፅንዓት፡ *እዚ ምርምር ፅንዓት ኣብ ሕብረተሰብ ዉሽጢ ዝተኸሰተ ናይ ቆርበት ኢንፌክሽን ዘለዎም ፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊ ዘለዎም፣ ኣብ ሕርሻ ፍርያት ፀባ ዝሰርሑ ሰባትን ኣብኡ ዝርከባ ኣላሕምን ዘካትት እዩ፡፡ ስለዚ ኣብዚ ምርምራዊ መፅናዕቲ ንክሳተፉ/ፋ ተጋቢዞም/ዘን ኣለዉ/ዋ፡፡*

ሽቶ እቲ ምርምር ፅንዓት፡ *ብስታፊሎኮከስ ኦውሪየስ ዝመፅእ ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባ ንምፍላጥ፤ ነዞም ዝተጠቐሱ ሕማማት ዘምፅኡ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ሥርጭቶምን ንምርዳእ፤ ከምኡዉን እዚ ባክተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት ንምፍላጥ እዩ፡፡*

እቲ ምርምር ፅንዓት ዝወስዶ ግዘ፡ ክሳብ ኣርባዕተ ኣዋርሕ ክወስድ ተባሂሉ ይግመት፡፡ ይኹን እምበር ካባኹም/ካብአን ዝዉሰድ ናይ ላቦራቶሪ ናሙና ሓደ ግዘ ጥራሕ እዩ፡፡

ቅደም ተከተል ኣተገባብራ እቲ ፅንዓት፡ መጀመርያ ነቲ ፅንዓት ዝምልከቱ ውሑዳት ሕቶታት ክጥየቑ እዮም፡፡ ብምቕፃል ካብቲ እንፌክሽን ዘለዎ ናይ ቖርበቶም ክፍሊ ንላቦራቶሪ ምርመራ ዝውዕል ናሙና ብዝሰልጠነ/ት ነርስ ይዉሰድ፡፡ እቲ ናሙና ንምርመራ ናብ ላቦራቶሪ ይዉሰድሞ ስታፊሎኮከስ ኦውሪየስ እንተተረኺቡ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ይኸይድ፡፡

ኣብቲ ምርምር ፅንዓት እንትሳተፉ ከጋጥም ዝኽእል ፀገም፡ ናይ ላቦራቶሪ ናሙና ኣብ ዝዉሰደሉ እዋን ብጣዕሚ ንኡሽተይ ሕማም ክስመዖም/ዐን ይኽእል እዩ፡፡ ነዚ ይ ንምቕራፍ እቲ ናሙና ብዝሰልጠነ/ት ነርስ ብጥንቃቐ ክዉሰድ እዩ፡፡

ረብሓ፡ ኹሉ ናይ ላቦራቶሪ ምርመራ ብነፃ ዝግበር እንትኸዉን ዉፅኢት ናይቲ ምርመራ ዝሓሸ ሕክምና መአንታን ክረኽቡ ንዝከታተሎም ሓኪም ይወሃብ፡፡ ኣብዚ ምርምር ፅንዓት ብምስታፎም/ክን ካበኦምን ካብ ካልኦትን እንረኽቦ መረዳእታ ብስታፊሎኮከስ ኦውሪየስ ምኽንያት ዝመፅእ ናይ ቆርበት ኢንፌክሽን መከላኸልን መቆፃፀርን ሜላ ንምንዳፍ ዓብዪ ረብሓ አለዎ፡፡

ሚስጢራዊነት፡ ክብርን ሚስጢርን ተሳተፍቲ ናይዚ ፅንዓት ብዘተኣማምን ከምንሕሉ ክነረጋግፅ ንፎቱ፡፡ መንነቶም/ክን ዝሕብር ምንም ዓይነት መረዳእታ ካብቲ ናይ ምርምር ጉጅለ ወፃኢ ንማንም ኣይነካፍልን፡፡ እቲ መፅናዕቲ እብ ዝሕተመሉ እዋን ሽምኩም/ን ኣይፀሓፍን፡፡ ካባኹም/ን/ እንረኽቦ መረዳእታ ኣብ ዝቑለፍ ሳንዱቕ ብጥንቃቐ ዝቕመጥ እንትኸዉን ኣብ ኮምፒውተር ዝቕመጥ መረዳእታ ድማ ብፓስዎርድ ይቑለፍ፡፡

ናይ ምስታፍ ፈቃደኝነት፡ ኣብዚ ፅንዓት ምስታፍ ሙሉእ ብሙሉእ ኣብ ፈቓዶም/ድክን ዝተመስረተ እንትኸዉን ናይ ዘይምስታፍ መሰልኹም/ኽን ዝተሓለወ እዩ፡፡ ዋላ ድሕሪ ፍቓድ ምሃብኩም/ክን ኣብዝኾነ ግዘ ካብቲ መፅናዕቲ ክግለሉ/ላ ይኽክሉ/ላ እዮም/የን፡፡ እዚ ዉሳነ ንሐዚ ወይ ድማ ንቐፃሊ ኣብዚ ጥዕና ተቋም ንዝረኽብዎ ኣገልግሎት ሕክምና ዝፈጥሮ ፀገም ኣይህልን፡፡

እድራሻ፡ ኣብዚ ፅንዓት ሕቶ እንተልይዎም/ወን ሐዚ ምጥያቕ ዝኽእሉ/ላ እንትኾኑ/ና ተወሳኺ መብራህርሂ እንተደልዮም/ን ድማ ነዞም ዝቕፅሉ ተመራመርትን ስነምግባር ምርምር ኮሚቴን የዘራርቡ/ባ፡፡ አለም አብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡ 0911225832)፣ ናይ አዲስ አበባ ዩኒቨርሲቲ ኮሚቴ ስነምግባር ምርምር (ስልክ 0118961396)

Annex I-B: Information sheet for dairy farm workers

I-B: English Version

Title of the project: Phenotypic and molecular characterization of *Staphylococcus aureus* from human and animals in Mekele, Northern Ethiopia

Principal investigator: Alem Abrha (BSc, MSc, PhD candidate, Medical Microbiology)

Organization: Addis Ababa University, college of Health Sciences, Department of Microbiology, Immunology and Parasitology

Sponsors: Addis Ababa University, the Ohio State University

Introduction: *S. aureus* is a commensal and dangerous pathogen capable of infecting virtually every tissue of the body. Skin and soft tissue infections in the community, and surgical site infections in the health care setting are mainly caused by this bacterium. Besides to human infections, *S. aureus* can also colonize and infect animals; where mastitis in dairy animals being the best example. Studies from abroad demonstrated that infected animals by *S. aureus* can act as a reservoir for zoonotic infections of humans. Despite its importance to public and animal health, data regarding molecular types, zoonotic transmission and toxin characteristics of *S. aureus* is lacking in Ethiopia.

Study participants: This study involves patients with community onset skin and soft tissue infections, postoperative wound infections at Ayder referral hospital; dairy cows and farm workers in and around Mekele. Therefore, you are kindly invited to participate in this study.

Purpose of the study: to determine the burden and drug resistance pattern of *Staphylococcus aureus* from community onset skin and soft tissue infections, post-operative wound infections, bovine mastitis and dairy farm workers; to understand the molecular types of *S. aureus* isolates and their virulence characteristics in the study setting.

Duration: the duration of this study depends on the availability of study participants; but it is estimated that it can take about four months. However, laboratory sample from you will be collected only once.

Procedure: the procedure is easy and simple; first you will be asked few questions and then nasal swab will be collected with great care. The sample will be transported to Laboratory for analysis. If *S. aureus* is isolated, it will be transported to abroad for further investigation.

Risk and discomfort: You may feel minor pain during sample (nasal swab) collection. However, to minimize discomfort; the sample will be collected by trained professional with appropriate precaution.

Expected benefits: The laboratory investigations will be performed free of charge. The information gained from you and others will help to consider *S. aureus* infection prevention and control strategy at local or national level.

Confidentiality: We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. If a research article or publication comes from this study, you will not be identified by name. The information we collect as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

Voluntary Participation and Withdrawal: Your participation is completely voluntary and you have the right not to participate in this study. You can stop yourself from participating in the study at any time after giving your consent. This decision will not affect you by any means.

Contact information: If you have any questions about this study you can ask now or contact the following investigators and the ethics committee for further information. Alem Abrha (Tel 0911784037); Dr. Daniel Asrat (Tel 0911223019); Dr. Yimtubezenash Woldeamanuel (Tel 0911225832); IRB-CHS (Tel : +251-118961396).

I-B: Information Sheet (Amharic Version)

<u>አማርኛ ቅጅ</u>

የጥናቱ ርእስ፡ ‘ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ አኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ’

የዋና ተመራማሪ ሥም፡ አለም አብርሃ (በሜዲካል ማይክሮባዮሎጂ የሦስተኛ ዲግሪ ተማሪ)

የድርጅት ሥም፡ አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ የማይክሮባዮሎጂ ኢሙኖሎጂ እና ፓራሲቶሎጂ ት/ት ክፍል

ዋና ስፖንሰር፡ አዲስ አበባ ዩኒቨርሲቲ ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ

መግቢያ፡ ስታፊሎኮከስ ኦውሪየስ በተለያዩ የሰዉንታችን ክፍሎች የሚገኝና በየትኛዉም የሰዉነታችን ክፍል በሽታ ማስከተል የሚችል ባክተርያ ነው፡፡ ስታፊሎኮከስ ኦውሪየስ የቆዳ እና የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን በማምጣት ግንባር ቀደም ነው፡፡ ይህ ባክተርያ ከሰው አልፎ በተለያዩ እንስሳት የተለያዩ የበሽታ ዓይነቶችን በማምጣት የሚታወቅ ሲሆን፤ ከነዚህ መካከል በወተት ላሞች የጡት ኢነፍክሽን (ማስታይተስ) ዋነኛው ነው፡፡ በባክተርያው የተጠቁት እንስሳት ባክተርያዉን ወደ ሰው ማስተላለፍ እንደሚችሉ ከአገራችን ዉጭ የተሰሩ ጥናቶች ያመላክታሉ፡፡ ስታፊሎኮከስ ኦውሪየስ በሰውና እንስሳት የሚያመጣው የጤና ችግር ከፍተኛ ቢሆንም በአገራችን በዚህ ባክተርያ ዙርያ ያለው በምርምር የተደገፈ መረጃ ውስን ነው፡፡ በተለይ በአገራችን ያሉትን የስታፊሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸው፣ ባከተርያው በሰውና እንስሳት መሃከል ሰለመተላለፉ እና ባክተርያው የያዛቸው መርዛማ ነገሮች (ቶክሲንስ) ምንም ዓይነት መረጃ የለም፡፡

የጥናቱ ተሳታፊዎች፡ ይህ ጥናት በህብረተሰብ ዉስጥ የተከሰተ የቆዳ ኢንፌክሽን ያላቸው፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን ያላቸው ፤ እንዲሁም በወተት እርሻ ዉስጥ የሚገኙ ሰራተኞችና ላሞች ያካትታል፡፡ በመሆኑም በዚህ ጥናት እንዲሳተፉ በአክብሮት ተጋብዘዋል፡፡

የጥናቱ ዓላማ፡ በስታፊሎኮከስ ኦውሪየስ የሚመጣዉን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የላሞች የጡት እንፌክሽን ለማወቅ፤ እነዚህን በሽታዎችን ያመጡ የስታፊሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸዉን ለመረዳት እና ባክተርያው በሰውና እንስሳት መሃከል ያለዉን የመተላለፍ ሁኔታ ለማወቅ ነው፡፡

ጥናቱ የሚወስደው ጊዜ፡ ይህ ጥናት እስከ አራት ወራት ይወስዳል ተብሎ ይገመታል፡፡ ይሁን እንጂ ከእርስዎ የሚወሰደው የላቦራቶሪ ናሙና አንዴ ብቻ ነው፡፡

በጥናቱ የሚከናወኑ ተግባራት በቅደም ተከተል፡ በቅድምያ ጥቂት ጥያቄዎች ይጠየቃሉ፡፡ በመቀጠልም ከአፍንጫዎ ዉስጥ የላቦራቶሪ ናሙና በጥንቃቄ ይወሰዳል፡፡ ናሙናው ለምርመራ ወደ ላቦራቶሪ ይሄድና ስታፊሎኮከስ ኦውሪየስ ከተገኘ ለተጨማሪ ምርመራ ወደ ዉጭ ሃገር ይሄዳል፡፡

በጥናቱ ሲሳተፉ ሊፈጠር የሚችል ችግር፡ ከአፍንጫዎ ናሙና በሚወሰድበት ጊዜ በጣም አነስተኛ ህመም ሊኖር ይችላል፡፡ ነገር ግን ይህንን ለመቀነስ ናሙናዉን በሰለጠነ ባለሞያ በጥንቃቄ ይወሰዳል፡፡

የጥናቱ ጠቀሜታ፡ የላቦራቶሪ ምርመራው በነፃ የሚደረግ ነው፡፡ በዚህ ጥናት በመሳተፍዎ ከእርስዎ እና ከሌሎች የሚገኘው መረጃ በአገራችን በስታፊሎኮከስ ኦውሪየስ አማካኝነት የሚከሰተዉን የቆዳ ኢንፌክሽን የመከላከያና የቁጥጥር ዘዴ ለመንደፍ ትልቅ ጠቀሜታ ይኖረዋል፡፡

ሚስጢራዊነት፡ የእርስዎ ክብርና ሚሰጢር በአስተማማኝ ሁኔታ እንጠብቃለን፡፡ እርስዎ/ልጅዎ የሚለይ መረጃ ከጥናት ቡደኑ ዉጭ ለማንም አናካፍልም፡፡ የጥናቱ ዉጤት ሲታተም የእርስዎ ሥም አይፃፍም፡፡ የምንሰበስበው መረጃ ቁልፍ ባለው ሳጥን የሚቀመጥ ሲሆን በኮምፒውተር የሚቀመጠዉን መረጃ ደገሞ በፓስዎረድ ይቆለፋል፡፡

የተሳትፎ ፈቃደኝነት፡ በዚህ ጥናት መሳተፍ ሙሉበሙሉ በፈቃደኝት የተመሰረተ ሲሆን ያለመሳተፍ መብትዎ የተጠበቀ ነው፡፡ ፈቃደኝነትዎ ከሰጡ ቡኃላም ቢሆን በማነኛዉም ሰዓት ከጥናቱ ራስዎን ማግለል ይችላሉ፡፡ እነዲህ በመወሰንዎ በምንም አይነት መልኩ የሚፈጥርብዎ ተፅዕኖ የለም፡፡

አድራሻ፡ በጥናቱ ዙርያ ማነኛዉም ዓይነት ጥያቄ አሁን መጠየቅ የሚችሉ ሲሆን ተጨማሪ ማብራርያ ቢፈልጉ ለሚከተሉት ተመራማሪዎችና የሥነምግባር ኮሚቴ በስልክ ማናገር ይችላሉ፡፡ አለም አብርሃ (ስልክ፡ 0911784037) ፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡ 0911225832)፣ የአዲስ አበባ ዩኒቨርሲቲ የምርምር ስነምግባር ኮሚቴ (ስልክ፡ 0118961396)

I-B: Information sheet (Tigrigna version)

<u>ትግርኛ ቕዳሕ</u>

አርእስቲ ምርምር ፅንዓት፡ *'ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ አኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ'*

ሽም ዋና ተመራማሪ፡ *ኣለም ኣብርሃ (ብሜዲካል ማይክሮባዮሎጂ ተምሃራይ ሳልሳይ ዲግሪ)*

ሽም ድርጅት፡ *ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኮሌጅ ጥዕና ሳይንስ ፣ ክፍሊ ት/ቲ ማይክሮባዮሎጂ ኢሙኖሎጂን ፓራሲቶሎጂን*

ዋና ስፖንሰር፡ *ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ*

መእተዊ፡ *ስታፊሎኮከስ ኦውሪየስ ኣብ ዝተፈላለዩ ኣካላት ሰዉነትና ዝርከብን ኣብ ኩሉ ክፍሊ ኣካላት ሰዉነትና ኢንፌክሽን ከምፅእ ዝኽእል ባክተርያ እዩ፡፡ ስታፊሎኮከስ ኦውሪየስ ኢንፌክሽን ቆርበትን ድሕሪ-መጥባሕቲ ቑስልን ብምምፃእ ቀዳማይ እዩ፡፡ እዚ ባክተርያ ካብ ሰብ ወፃኢ ኣብ ዝተፈላለዩ እንስሳታት ኢንፌክሽን ዘምፅእ እንትኾን፤ ኣብ ናይ ፀባ ከፍቲ ኢነፍክሽን ጡብ (ማስታይተስ) ብምምፃእ ይፍለጥ፡፡ ብስታፊሎኮከስ ኦውሪየስ ዝተጠቕዑ እንስሳት እቲ ባክተርያ ናብ ሰብ ምምሕልላፍ ከምዝኽእሉ ካብ ሃገርና ወፃኢ ዝተሰርሑ ምርምር ፅንዓታት የረድኡ፡፡ ስታፊሎኮከስ ኦውሪየስ ኣብ ሰብን እንስሳታትን ዘምፅኦ ሳዕቤን ጥዕና ዓብዪኳ እንተኾነ ኣብ ሃገርና ኣብዚ ባክተርያ ዘሎ ብምርምር ዝተደገፈ ሓበሬታ ውስን እዩ፡፡ ብፍላይ ኣብ ሃገርና ዘለዉ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ስርጭቶምን፣ እዚ ባክተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት፣ እዚ ባክተርያ ዝተሸከሞም ኩነታት መርዛማ ነገራት (ቶክሲንስ) ምንም ዓይነት መረዳእታ የለን፡፡*

ተሳተፍቲ ምርምር ፅንዓት፡ *እዚ ምርምር ፅንዓት ኣብ ሕብረተሰብ ዉሽጢ ዝተኸሰተ ናይ ቆርበት ኢንፌክሽን ዘለዎም ፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊ ዘለዎም፣ ኣብ ሕርሻ ፍርያት ፀባ ዝሰርሑ ሰባትን ኣብኡ ዝርከባ ኣላሕምን ዘካትት እዩ፡፡ ስለዚ ኣብዚ ምርምራዊ መፅናዕቲ ንክሳተፉ/ፋ ተጋቢዞም/ዘን ኣለዉ/ዋ፡፡*

ሽቶ እቲ ምርምር ፅንዓት፡ *ብስታፊሎኮከስ ኦውሪየስ ዝመፅእ ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባ ንምፍላጥ፤ ነዞም ዝተጠቐሱ ሕማማት ዘምፅኡ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ሥርጭቶምን ንምርዳእ፤ ከምኡዉን እዚ ባክተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት ንምፍላጥ እዩ፡፡*

እቲ ምርምር ፅንዓት ዝወስዶ ግዜ፡ ክሳብ ኣርባዕተ ኣዋርሕ ክወስድ ተባሂሉ ይግመት፡፡ ይኹን እምበር ካባኹም/ካብአን ዝዉሰድ ናይ ላቦራቶሪ ናሙና ሓደ ግዘ ጥራሕ እዩ፡፡

ቅደም ተከተል ኣተገባብራ እቲ ፅንዓት፡ መጀመርያ ነቲ ፅንዓት ዝምልከቱ ውሑዳት ሕቶታት ክጥየቑ/ቓ እዮም/የን፡፡ ብምቕፃል ካብ አፍንጫኦም/አን ብጥንቃቐ ናይ ላቦራቶሪ ናሙና ልምዲ ብዘለዎ በዓል ሞያ ይዉሰድ፡፡ እቲ ናሙና ንምርመራ ናብ ላቦራቶሪ ይዉሰድሞ ስታፊሎኮከስ ኦውሪየስ እንተተረኺቡ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ይኸይድ፡፡

ኣብቲ ምርምር ፅንዓት እንትሳተፉ ከጋጥም ዝኽእል ፀገም፡ ናይ ላቦራቶሪ ናሙና ኣብ ዝዉሰደሉ እዋን ብጣዕሚ ንኡሽተይ ሕማም ክስመየም/ዐን ይኽእል እዩ፡፡ ነዚ ይ ንምቕራፍ እቲ ናሙና ልምዲ ብዘለዎ በዓል ሞያ ብጥንቃቐ ክዉሰድ እዩ፡፡

ረብሓ፡ ኹሉ ናይ ላቦራቶሪ ምርመራ ብነፃ ዝግበር እዩ፡፡ ኣብዚ ምርምር ፅንዓት ብምስታፎም/ክን ካበኦምን ካብ ካልኦትን እንረኽቦ መረዳእታ ብስታፊሎኮከስ ኦውሪየስ ምክንያት ዝመፅእ ናይ ቆርበት ኢንፌክሽን መከላኸልን መቆፃፀርን ሜላ ንምንዳፍ ዓብዪ ረብሓ አለዎ፡፡

ሚስጢራዊነት፡ ክብርን ሚስጢርን ተሳተፍቲ ናይዚ ፅንዓት ብዘተኣማምን ከምንሕሉ ክነረጋግፅ ንፎቱ፡፡ ናቶም/ናትክን መንነት ዝሕብር ምንም ዓይነት መረዳእታ ካብቲ ናይ ምርምር ጉጅለ ወፃኢ ንማንም ኣይነካፍልን፡፡ እቲ መፅናዕቲ እብ ዝሕተመሉ እዋን ሽምኩም/ን ኣይፀሓፍን፡፡ ካባኹም/ን እንረኽቦ መረዳእታ ኣብ ዝቑለፍ ሳንዱቕ ብጥንቃቐ ዝቕመጥ እንትኾዉን ኣብ ኮምፒውተር ዝቕመጥ መረዳእታ ድማ ብፓስዎርድ ይቑለፍ፡፡

ናይ ምስታፍ ፈቃደኘነት፡ ኣብዚ ፅንዓት ምስታፍ ሙሉእ ብሙሉእ ኣብ ፈቓዶም/ድክን ዝተመስረተ እንትኾዉን ናይ ዘይምስታፍ መሰልኹም/ኽን ዝተሓለወ እዩ፡፡ ዋላ ድሕሪ ፍቓድ ምሃብኩም/ክን ኣብዝኾነ ግዘ ካብቲ መፅናዕቲ ክግልሉ/ላ ይኽክሉ/ላ እዮም/የን፡፡ እዚ ዉሳነ ብዝኾነ ዓይነት መንገዲ ዝፈጥረሎም/ለን ፀገም ኣይህልን፡፡

እድራሻ፡ ኣብዚ ፅንዓት ሕቶ እንተልይዎም/ክን ሐዚ ምጥያቕ ዝኽእሉ/ላ እንትኾኑ/ና ተወሳኺ መብራህርሂ እንተደልዮም/የን ድማ ነዞም ዝቕፅሉ ተመራመርትን ስነምግባር ምርምር ኮሚቴን የዘራርቡ/ባ፡፡ አለም አብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡ 0911225832)፣ ናይ አዲስ አበባ ዩኒቨርሲቲ ኮሚቴ ስነምግባር ምርምር (ስልክ: 0118961396)

Annex I-C: Information sheet for Parents/guardians

I-C: English Version

Title of the project: Phenotypic and molecular characterization of *Staphylococcus aureus* from human and animals in Mekele , Northern Ethiopia

Principal investigator: Alem Abrha (BSc, MSc, PhD fellow in Medical Microbiology)

Organization: Addis Ababa University, college of Health Sciences, Department of Microbiology, Immunology and Parasitology

Sponsors: Addis Ababa University, the Ohio State University

Introduction: *S. aureus* is a commensal and dangerous pathogen capable of infecting virtually every tissue of the body. Skin and soft tissue infections in the community, and surgical site infections in the health care setting are mainly caused by this bacterium. Besides to human infections, *S. aureus* can also colonize and infect animals; where mastitis in dairy animals being the best example. Studies from abroad demonstrated that infected animals by *S. aureus* can act as a reservoir for zoonotic infections of humans. Despite its importance to public and animal health, data regarding molecular types, zoonotic transmission and toxin characteristics of *S. aureus* is lacking in Ethiopia.

Study participants: This study involves patients with community onset skin and soft tissue infections, postoperative wound infections at Ayder referral hospital; dairy cows and farm workers in and around Mekele. Therefore, you are kindly invited to let your child participate in this study.

Purpose of the study: to determine the burden and drug resistance pattern of *Staphylococcus aureus* from community onset skin and soft tissue infections, post-operative wound infections, bovine mastitis and dairy farm workers; to understand the molecular types of *S. aureus* isolates and their virulence characteristics in the study setting.

Duration: the duration of this study depends on the availability of study participants; but it is estimated that it can take about four months. However, laboratory sample from your child will be collected only once.

Procedure: the procedure is easy and simple; first you will be asked few questions and then wound swab/ pus sample will be collected from your child by trained nurse. The sample will be transported to Laboratory for analysis. If *S. aureus* is isolated, it will be transported to abroad for further investigation.

Risk and discomfort: Your child may feel minor discomfort during sample collection. However, to minimize discomfort; sample will be collected by trained nurse following appropriate precautions.

Expected benefits: The laboratory investigations will be performed free of charge and results will be communicated with your child's physician for better medical care. Besides the direct benefit your child can get, the information gained from him/her and others will help to consider *S. aureus* infection prevention and control strategy at local or national level.

Confidentiality: We respect your child's privacy and confidentiality. Any information that identifies your child will not be shared with anyone else outside the study team. If a research article or publication comes from this study, your child will not be identified by name. The information we collect as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

Voluntary Participation and Withdrawal: The participation is completely voluntary and you have the right not to let your child participate in this study. You can stop your child from participating in the study at any time after giving your consent. This decision will not affect in any way your child's current or future medical care in the health facility.

Contact information: If you have any questions about this study you can ask now or contact the following investigators and the ethics committee for further information. Alem Abrha (Tel 0911784037); Dr. Daniel Asrat (Tel 0911223019); Dr. Yimtubezenash Woldeamanuel (Tel 0911225832); IRB-CHS (Tel : +251-118961396).

I-C: Information sheet: Amharic Version

<u>**አማርኛ ቅጅ**</u>

የጥናቱ ርእስ፡ ‘ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ አኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ’

የዋና ተመራማሪ ሥም፡ አለም አብርሃ (በሜዲካል ማይክሮባዮሎጂ የሦስተኛ ዲግሪ ተማሪ)

የድርጅትሥም፡ አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ የማይክሮባዮሎጂ ኢሙኖሎጂ እና ፓራሲቶሎጂ ት/ት ክፍል

ስፖንሰር፡ አዲስ አበባ ዩኒቨርሲቲ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ

መግቢያ፡ ስታፊሎኮከስ ኦውሪየስ በተለያዩ የሰዉንታችን ክፍሎች የሚገኝና በየትኛዉም የሰዉነታችን ክፍል በሽታ ማስከተል የሚችል ባክተርያ ነው፡፡ ስታፊሎኮከስ ኦውሪየስ የቆዳ እና የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን በማምጣት ግንባር ቀደም ነው፡፡ ይህ ባክተርያ ከሰው አልፎ በተለያዩ እንስሳት የተለያዩ የበሽታ ዓይነቶችን በማምጣት የሚታወቅ ሲሆን፤ ከነዚህ መካከል በወተት ላሞች የጡት ኢነፍክሽን (ማስታይተስ) ዋነኛው ነው፡፡ በባከተርያው የተጠቁት እንስሳት ባክተርያዉን ወደ ሰው ማስተላለፍ እንደሚችሉ ከአገራችን ዉጭ የተሰሩ ጥናቶች ያመላክታሉ፡፡ ስታፊሎኮከስ ኦውሪየስ በሰውና እንስሳት የሚያመጣው የጤና ችግር ከፍተኛ ቢሆንም በአገራችን በዚህ ባክተርያ ዙርያ ያለው በምርምር የተደገፈ መረጃ ውስን ነው፡፡ በተለይ በአገራችን ያሉትን የስታፊሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸው፣ ባከተርያው በሰውና እንስሳት መሃከል ሰለመተላለፉ እና ባክተርያው የያዛቸው መርዛማ ነገሮች (ቶክሲንስ) ምንም ዓይነት መረጃ የለም፡፡

የጥናቱ ተሳታፊዎች፡ ይህ ጥናት በህብረተሰብ ዉስጥ የተከሰተ የቆዳ ኢንፌክሽን ያላቸው፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን ያላቸው ፤ እነዲሁም በወተት እርሻ ዉስጥ የሚገኙ ሰራተኞችና ላሞች ያካትታል፡፡ በመሆኑም በዚህ ጥናት ልጅዎ እነዲሳተፍ እነዲፈቅዱ በአክብሮት ተጋብዘዋል፡፡

የጥናቱ ዓላማ፡ በስታፊሎኮከስ ኦውሪየስ የሚመጣዉን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የላሞች የጡት እንፌክሽን ለማወቅ፤ እነዚህን በሽታዎችን ያመጡ የስታፊሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸዉን ለመረዳት እና ባክተርያው በሰውና እንስሳት መሃከል ያለዉን የመተላለፍ ሁኔታ ለማወቅ ነው፡፡

ጥናቱ የሚወስደው ጊዜ፡ ይህ ጥናት በቆዳ ኢንፌክሽን ወደ ሆሰፒታሎቹ በሚመጡ ህምመተኞች ብዛት የሚወሰን ቢሆንም እሰከ አራት ወራት ይወስዳል ተብሎ ይገመታል፡፡ ይሁን እንጂ ከልጅዎ የሚወሰደው የላቦራቶሪ ናሙና አንዴ ብቻ ነው፡፡

በጥናቱ የሚከናወኑ ተግባራት በቅደም ተከተል፡ በቅድምያ ጥቂት ጥያቄዎች ይጠየቃሉ፡፡ በመቀጠልም ከልጅዎ ኢንፌክሽን ካለበት ቦታ የላቦራቶሪ ናሙና በሰለጠነ/ች ነርስ ይወሰዳል፡፡ ናሙናው ለምርመራ ወደ ላቦራቶሪ ይሄድና ስታፌሎኮከስ ኦውሪየስ ከተገኘ ለተጨማሪ ምርመራ ወደ ዉጭ ሃገር ይሄዳል፡፡

ለጅዎ በጥናቱ ሲሳተፍ/ስትሳተፍ ሊፈጠር የሚችል ችግር፡ የቆዳ ኢንፌክሽን ካለበት ቦታ ናሙና በሚወሰድበት ጊዜ በጣም አነስተኛ ህመም ሊኖር ይችላል፡፡ ነገር ግን ይህንን ለመቀነስ ናሙናዉን በሰለጠነ/ች ነርስ በጥንቃቄ ይወሰዳል፡፡

የጥናቱ ጠቀሜታ፡ የላቦራቶሪ ምርመራው በነፃ የሚደረግ ሲሆን ዉጤቱን ለልጅዎ የተሻለ ህክምና ይረዳዘንድ ለሚከታተለው/ላት ሀኪም ይሰጣል፡፡ በዚህ ጥናት መሳተፍ ለልጅዎ ከሚሰጠው/ጣት ቀጥተኛ ጥቅም በላይ ከልጅዎ እና ከሌሎች የሚገኘው መረጃ በአገራችን በስታፌሎኮከስ ኦውሪየስ አማካኝነት የሚከሰተዉን የቆዳ ኢንፌክሽን የመከላከያና የቁጥጥር ዘዴ ለመንደፍ ትልቅ ጠቀሜታ ይኖረዋል፡፡

ሚስጢራዊነት፡ የልጅዎ ክብርና ሚሰጢር በአስተማማኝ ሁኔታ እንጠብቃለን፡፡ ልጅዎ የሚለይ መረጃ ከጥናት ቡደኑ ዉጭ ለማንም አናካፍልም፡፡ የጥናቱ ዉጤት ሲታተም የልጅዎ ሥም አይፃፍም፡፡ የምንሰበስበው መረጃ ቁልፍ ባለው ሳጥን የሚቀመጥ ሲሆን በኮመፒውተር የሚቀመጠዉን መረጃ ደገሞ በፓስዎረድ ይቆለፋል፡፡

የተሳትፎ ፈቃደኝነት፡ በዚህ ጥናት መሳተፍ ሙሉበሙሉ በፈቃደኝት የተመሰረተ ሲሆን ልጅዎን ያለማሳተፍ መብትዎ የተጠበቀ ነው፡፡ ፈቃደኝነትዎ ከሰጡ ቡኃላም ቢሆን በማነኛዉም ስዓት ከጥናቱ ልጅዎን ማግለል ይችላሉ፡፡ እነዚህ በመወሰንዎ በአሁኑ ይሁን የወደፊት የልጅዎ ህክምና አገልግሎት በዚህ ጤና ተቋም የሚፈጥርብዎ ተፅዕኖ የለም፡፡

አድራሻ፡ በጥናቱ ዙርያ ማነኛዉም ዓይነት ጥያቄ አሁን መጠየቅ የሚችሉ ሲሆን ተጨማሪ ማብራርያ ቢፈልጉ ለሚከተሉት ተመራማሪዎችና የሥነምግባር ኮሚቴ በስልክ ማናገር ይችላሉ፡፡ አለም አብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡ 0911225832)፣ የአዲስ አበባ ዩኒቨርሲቲ የምርምር ስነምግባር ኮሚቴ (ስልክ፡ 0118961396)

I-C: Information sheet: Tigrigna Version

ትግርኛ ቕዳሕ

ኣርእስቲ ምርምር ፅንዓት፡ 'ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ሀዩማን ኤንድ ኣኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ'

ሽም ዋና ተመራማሪ፡ ኣለም ኣብርሃ (ብሜዲካል ማይክሮባዮሎጂ ተምሃራይ ሳልሳይ ዲግሪ)

ሽም ድርጅት፡ ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኮሌጅ ጥዕና ሳይንስ ፣ ክፍሊ ት/ቲ ማይክሮባዮሎጂ፣ ኢሙኖሎጂን ፓራሲቶሎጂን

ዋና ስፖንሰር፡ ዩኒቨርሲቲ ኣዲስ ኣበባ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ

መእተዊ፡ ስታፊሎኮከስ ኦውሪየስ ኣብ ዝተፈላለዩ ኣካላት ሰዉነትና ዝርከብን ኣብ ኩሉ ክፍሊ ኣካላት ሰዉነትና ኢንፌክሽን ከምፅእ ዝኽእል ባክተርያ እዩ፡፡ ስታፊሎኮከስ ኦውሪየስ ኢንፌክሽን ቀርበትን ድሕሪ-መጥባሕቲ ቑስልን ብምምፃእ ቀዳማይ እዩ፡፡ እዚ ባክተርያ ካብ ሰብ ወፃኢ ኣብ ዝተፈላሉዩ እንስሳታት ኢንፌክሽን ዘምፅእ እንትኾን፣ ኣብ ናይ ፀባ ከፍቲ ኢነፍክሽን ጡብ (ማስታይተስ) ብምምፃእ ይፍለጥ፡፡ ብስታፊሎኮከስ ኦውሪየስ ዝተጠቕዑ እንስሳት እቲ ባክተርያ ናብ ሰብ ምምሕልላፍ ከምዝኽእሉ ካብ ሃገርና ወፃኢ ዝተሰርሑ ምርምር ፅንዓታት የረድኡ፡፡ ስታፊሎኮከስ ኦውሪየስ ኣብ ሰብን እንስሳታትን ዘምፅኦ ሳዕቤን ጥዕና ዓብዪኳ እንተኾነ ኣብ ሃገርና ኣብዚ ባክተርያ ዘሎ ብምርምር ዝተደገፈ ሓበሬታ ውስን እዩ፡፡ ብፍላይ ኣብ ሃገርና ዘለዉ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ስርጭቶምን፣ እዚ ባከተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት፣ እዚ ባክተርያ ዝተሸከሞም ኩነታት መርዛማ ነገራት (ቶክሲንስ) ምንም ዓይነት መረዳእታ የለን፡፡

ተሳተፍቲ ምርምር ፅንዓት፡ እዚ ምርምር ፅንዓት ኣብ ሕብረተሰብ ዉሽጢ ዝተኽሰተ ናይ ቀርበት ኢንፌክሽን ዘለዎም ፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊ ዘለዎም፣ ኣብ ሕርሻ ፍርያት ፀባ ዝሰርሑ ሰባትን ኣብኡ ዝርከባ ኣላሕምን ዘካትት እዩ፡፡ ስለዚ ኣብዚ ምርምራዊ መፅናዕቲ ቆልያኦም/ኣን ክሳተፍ ንክፈቕዱ/ዳ ተጋቢዞም/ዘን ኣለዉ/ዋ፡፡

ሽቶ እቲ ምርምር ፅንዓት፡ ብስታፊሎኮከስ ኦውሪየስ ዝመፅእ ናይ ቀርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባ ንምፍላጥ፣ ነዞም ዝተጠቖሱ ሕማማት ዘምፀኡ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ሥርጭቶምን ንምርዳእ፣ ከምኡዉን እዚ ባክተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት ንምፍላጥ እዩ፡፡

እቲ ምርምር ፅንዓት ዝወስዶ ግዘ፡ ክሳብ ኣርባዕተ ኣዋርሕ ክወስድ ተባሂሉ ይግመት፡፡ ይኹን እምበር ካብ ቀልያኦም/ኣን ዝዉሰድ ናይ ላቦራቶሪ ናሙና ሓደ ግዘ ጥራሕ እዩ፡፡

ቅደም ተከተል ኣተገባብራ እቲ ፅንዓት፡ መጀመርያ ነቲ ፅንዓት ዝምልከቱ ውሑዳት ሕቶታት ክጥየቑ/ቓ እዮም/የን፡፡ ብምቕፃል ካብቲ እንፌክሽን ዘለዎ ናይ ቖርበት ክፍሊ ብጥንቃቐ ናይ ላቦራቶሪ ናሙና ብዝሰልጠነ/ት ነርስ ካብ ቀልያኦም ይዉሰድ፡፡ እቲ ናሙና ንምርመራ ናብ ላቦራቶሪ ይዉሰድሞ ስታፊሎኮከስ ኦውሪየስ እንተተረኺቡ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ይኸይድ፡፡

ቀልያኦም ኣብቲ ምርምር ፅንዓት እንትሳተፍ/ትሳተፍ ከጋጥም ዝኽእል ፀገም፡ ናይ ላቦራቶሪ ናሙና ኣብ ዝዉሰደሉ እዋን ብጣዕሚ ንኡሽተይ ሕማም ክስመየ/ዓ ይኽእል እዩ፡፡ ነዚ ይ ንምቕራፍ እቲ ናሙና ብዝሰልጠነ/ት ነርስ ብጥንቃቐ ክዉሰድ እዩ፡፡

ረብሓ፡ ኹሉ ናይ ላቦራቶሪ ምርመራ ብነፃ ዝግበር እንትኸዉን ዉፅኢት ናይቲ ምርመራ ዝሓሸ ሕክምና መእንታን ክረክብ/ክትረክብ ቀልያኦም ንዝከታተል ሓኪም ይወሃብ፡፡ ኣብዚ ምርምር ፅንዓት ክሳተፍ/ክትሳተፍ ብምግባሮም ካብ ቀልያኦምን ካብ ካልኦትን እንረኽቦ መረዳእታ ብስታፊሎኮከስ ኦውሪየስ ምኽንያት ዝመፅእ ናይ ቆርበት ኢንፌክሽን መከላኸልን መቆፃፀርን ሜላ ንምንዳፍ ዓብዪ ረብሓ ኣለዎ፡፡

ሚስጢራዊነት፡ ክብርን ሚስጢርን ተሳተፍቲ ናይዚ ፅንዓት ብዘተኣማምን ከምንሕሉ ክነረጋግፅ ንፎቱ፡፡ ናይ ቀልያኦም ማንነት ዝሕብር ምንም ዓይነት መረዳእታ ካብቲ ናይ ምርምር ጉጅለ ወፃኢ ንማንም ኣይነካፍልን፡፡ እቲ መፅናዕቲ እብ ዝሕተመሉ እዋን ሽም ቀልያኦም ኣይፀሓፍን፡፡ ካካብ ቀልያኦም እንረኽቦ መረዳእታ ኣብ ዝቑለፍ ሳንዱቕ ብጥንቃቐ ዝቕመጥ እንትኸዉን ኣብ ኮምፒውተር ዝቕመጥ መረዳእታ ድማ ብፓስዎርድ ይቑለፍ፡፡

ናይ ምስታፍ ፈቃደኝነት፡ ኣብዚ ፅንዓት ምስታፍ ሙሉእ ብሙሉእ ኣብ ፈቓዶም/ድክን ዝተመስረተ እንትኸዉን ቀልያኦም ክሳተፍ/ክትሳተፍ ናይ ዘይምግባር መሰልኹም/ኽን ዝተሓለወ እዩ፡፡ ዋላ ድሕሪ ፍቓድ ምሃብኩም/ክን ኣብዝኾነ ግዘ ቀልያኦም ካብቲ መፅናዕቲ ከግልሉ/ላ ይኽክሉ/ላ እዮም/የን፡፡ እዚ ዉሳነ ንሐዚ ወይ ድማ ንቸፃሊ ኣብዚ ጥዕና ተቋም ቀልያኦም ንዝረኽቦ/ንትረኽቦ ኣገልግሎት ሕክምና ዝፈጥሮ ፀገም ኣይህልን፡፡

ኣድራሻ፡ ኣብዚ ፅንዓት ሕቶ እንተልይዎም ሐዚ ምጥያቕ ዝኽእሉ እንትኾኑ ተወሳኺ መብራህርሂ እንተደልዮም ድማ ነዞም ዝቕፅሉ ተመራመርትን ስነምግባር ምርምር ኮሚቴን የዘራርቡ፡፡ ኣለም ኣብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል ኣስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡብዝናሽ ወ/ኣማኑኤል (ስልክ፡ 0911225832)፣ ናይ ኣዲስ ኣበባ ዩኒቨርሲቲ ኮሚቴ ስነምግባር ምርምር (ስልክ፡ 118961396)

Annex I-D: Information sheet for 12-18 years old study participants with skin and soft tissue infections

I-D: English Version

Title of the project: Phenotypic and molecular characterization of *Staphylococcus aureus* from human and animals in Mekele, Northern Ethiopia

Principal investigator: Alem Abrha (BSc, MSc, PhD candidate in Medical Microbiology)

Organization: Addis Ababa University; college of Health Sciences; Department of Microbiology, Immunology and Parasitology

Sponsors: Addis Ababa University**,** the Ohio State University

Introduction: *S. aureus* is a commensal and dangerous pathogen capable of infecting virtually every tissue of the body. Skin and soft tissue infections in the community, and surgical site infections in the health care setting are mainly caused by this bacterium. Besides to human infections, *S. aureus* can also colonize and infect animals; where mastitis in dairy animals being the best example. Studies from abroad demonstrated that infected animals by *S. aureus* can act as a reservoir for zoonotic infections of humans. Despite its importance to public and animal health, data regarding molecular types, zoonotic transmission and toxin characteristics of *S. aureus* are lacking in Ethiopia.

Study participants: This study involves patients with community onset skin and soft tissue infections, postoperative wound infections at Ayder referral hospital; dairy cows and farm workers in and around Mekele. Therefore, you are kindly invited to participate in this study.

Purpose of the study: to determine the burden and drug resistance pattern of *Staphylococcus aureus* from community onset skin and soft tissue infections, post-operative wound infections, bovine mastitis and dairy farm workers; to understand the molecular types of *S. aureus* isolates and their virulence characteristics in the study setting.

Duration: the duration of this study depends on the availability of study participants; but it is estimated that it can take about four months. However, laboratory sample from you will be collected only once.

Procedure: the procedure is easy and simple; first you will be asked few questions and then wound swab/ pus sample will be collected by trained nurse. The sample will be transported to laboratory for analysis. If *S. aureus* is isolated, it will be transported to abroad for further investigation.

Risk and discomfort: You may feel minor discomfort during sample collection. However, to minimize discomfort; collection will be performed by trained nurse following appropriate precaution.

Expected benefits: The laboratory investigations will be performed free of charge and results will be communicated with your physician; so that, you can get appropriate medical care. Besides the direct benefit you can get, the information gained from you and others will help to consider *S. aureus* infection prevention and control strategy at local or national level.

Confidentiality: We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. If a research article or publication comes from this study, you will not be identified by name. The information we collect as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

Voluntary Participation and Withdrawal: The participation is completely voluntary and you have the right not to participate in this study. You can stop yourself from participating in the study at any time after giving your consent. This decision will not affect in any way your current or future medical care in the health facility.

Contact information: If you have any questions about this study you can ask now or contact the following investigators and the ethics committee for further information. Alem Abrha (Tel 0911784037); Dr. Daniel Asrat (Tel 0911223019); Dr. Yimtubezenash Woldeamanuel (Tel 0911225832); IRB-CHS (Tel : +251-118961396).

I-D: Information sheet Amharic Version

<u>አማርኛ ቅጅ</u>

የጥናቱ ርእስ፡ *'ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፌሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ አኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ'*
የዋና ተመራማሪ ሥም፡ *አለም አብርሃ (በሜዲካል ማይክሮባዮሎጂ የሦስተኛ ዲግሪ ተማሪ)*
የድርጅትሥም፡ *አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ የማይክሮባዮሎጂ ኢሙኖሎጂ እና ፓራሲቶሎጂ ት/ት ክፍል*
ዋና ስፖንሰር፡ *አዲስ አበባ ዩኒቨርሲቲ ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ*

መግብያ፡ *ስታፌሎኮከስ ኦውሪየስ በተለያዩ የሰዉንታችን ክፍሎች የሚገኝና በየትኛዉም የሰዉነታችን ክፍል በሽታ ማስከተል የሚችል ባክተርያ ነው፡፡ ስታፌሎኮከስ ኦውሪየስ የቆዳ እና የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን በማምጣት ግንባር ቀደም ነው፡፡ ይህ ባክተርያ ከሰው አልፎ በተለያዩ እንስሳት የተለያዩ የበሽታ ዓይነቶችን በማምጣት የሚታወቅ ሲሆን፤ ከነዚህ መካከል በወተት ላሞች የጡት ኢነፍክሽን (ማስታይተስ) ዋነኛው ነው፡፡ በባከተርያው የተጠቁት እንስሳት ባክተርያዉን ወደ ሰው ማስተላለፍ እንደሚችሉ ከአገራችን ዉጭ የተሰሩ ጥናቶች ያመላክታሉ፡፡ ስታፌሎኮከስ ኦውሪየስ በሰውና እንስሳት የሚያመጣው የጤና ችግር ከፍተኛ ቢሆንም በአገራችን በዚህ ባክተርያ ዙርያ ያለው በምርምር የተደገፈ መረጃ ውስን ነው፡፡ በተለይ በአገራችን ያሉትን የስታፌሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸው፣ ባከተርያው በሰውና እንስሳት መሃከል ሰለመተላለፉ እና ባክተርያው የያዛቸው መርዛማ ነገሮች (ቶክሲንስ) ምንም ዓይነት መረጃ የለም፡፡*

የጥናቱ ተሳታፊዎች፡ *ይህ ጥናት በህብረተሰብ ዉስጥ የተከሰተ የቆዳ ኢንፌክሽን ያላቸው፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን ያላቸው ፣ እነዲሁም በወተት እርሻ ዉስጥ የሚገኙ ሰራተኞችና ላሞች ያካትታል፡፡ በመሆኑም በዚህ ጥናት እንድትሳተፍ/ፊ በአክብሮት ተጋብዘሃል/ሻል፡፡*

የጥናቱ ዓላማ፡ *በስታፌሎኮከስ ኦውሪየስ የሚመጣዉን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የላሞች የጡት እንፌክሽን ለማወቅ፤ እነዚህን በሽታዎችን ያመጡ የስታፌሎኮከስ ኦውሪየስ ዓይነቶችና ሥርጭታቸዉን ለመረዳት እና ባክተርያው በሰውና እንስሳት መሃከል ያለዉን የመተላለፍ ሁኔታ ለማወቅ ነው፡፡*

ጥናቱ የሚወስደው ጊዜ፡ ይህ ጥናት በቆዳ ኢንፌክሽን ወደ ሆስፒታሎቹ በሚመጡ ህምመተኞች ብዛት የሚወሰን ቢሆንም እስከ አራት ወራት ይወስዳል ተብሎ ይገመታል፡፡ ይሁን እንጂ ካንተ/ካነቺ የሚወሰደው የላቦራቶሪ ናሙና አንዴ ቢቻ ነው፡፡

በጥናቱ የሚከናወኑ ተግባራት በቅደም ተከተል፡ በቅድምያ ጥቂት ጥያቄዎች ትጠየቃለህ/ቂያለሽ፡፡ በመቀጠልም ኢንፌክሽኑ ካለበት ቦታ የላቦራቶሪ ናሙና በሰለጠነ/ች ነርስ ይወሰዳል፡፡ ናሙናው ለምርመራ ወደ ላቦራቶሪ ይሄድና ስታፊሎኮከስ ኦውሪየስ ከተገኘ ለተጨማሪ ምርመራ ወደ ዉጭ ሃገር ይሄዳል፡፡

በጥናቱ ሲሳተፉ ሊፈጠር የሚችል ችግር፡ የቆዳ ኢንፌክሽን ካለበት ቦታ ናሙና በሚወሰድበት ጊዜ በጣም አነስተኛ ህመም ሊኖር ይችላል፡፡ ነገር ግን ይህንን ለመቀነስ ናሙናዉን በሰለጠነ/ች ነርስ በጥንቃቄ ይወሰዳል፡፡

የጥናቱ ጠቀሜታ፡ የላቦራቶሪ ምርመራው በነፃ የሚደረግ ሲሆን ዉጤቱን ለተሻለ ህክምና ይረዳህ/ሽ ዘንድ ለሚከታተልህ/ሽ ሀኪም ይሰጣል፡፡ በዚህ ጥናት በመሳተፍህ/ሽ ላንተ/ቺ ከሚሰጠው ቀጥተኛ ጥቅም በላይ ካንተ/ቺ እና ከሌሎች የሚገኘው መረጃ በአገራችን በስታፊሎኮከስ ኦውሪየስ አማካኝነት የሚከሰተዉን የቆዳ ኢንፌክሽን የመከላከያና የቁጥጥር ዘዴ ለመንደፍ ትልቅ ጠቀሜታ ይኖረዋል፡፡

ሚስጢራዊነት፡ ያንተ/ቺ ክብርና ሚሰጢር በአስተማማኝ ሁኔታ እንጠብቃለን፡፡ አንተ/ቺን የሚለይ መረጃ ከጥናት ቡደኑ ዉጭ ለማንም አናካፍልም፡፡ የጥናቱ ዉጤት ሲታተም ያንተ/ቺ ሥም አይፃፍም፡፡ የምንሰበስበው መረጃ ቁልፍ ባለው ሳጥን የሚቀመጥ ሲሆን በኮመፒውተር የሚቀመጠዉን መረጃ ደገሞ በፓስዎረድ ይቆለፋል፡፡

የተሳትፎ ፈቃደኝነት፡ በዚህ ጥናት መሳተፍ ሙሉበሙሉ በፈቃደኝት የተመሰረተ ሲሆን ያለመሳተፍ መብትህ/ሽ የተጠበቀ ነው፡፡ ፈቃደኝነትህ/ሽ ከሰጠህ/ሽ ቡኃላም ቢሆን በማነኛዉም ሰዓት ከጥናቱ ማግለል ትችላለህ/ያለሽ፡፡ እነዲህ በመወሰንህ/ሽ በአሁኑ ይሁን ለወደፊቱ በዚህ ጤና ተቋም የምታገኘው/ኘው የህክምና አገልግሎት የሚፈጥረው ተፅዕኖ የለም፡፡

አድራሻ፡ በጥናቱ ዙርያ ማነኛዉም ዓይነት ጥያቄ አሁን መጠየቅ የምትችል/ዪ ሲሆን ተጨማሪ ማብራርያ ካስፈለገህ/ካስፈለገሽ ለሚከተሉት ተመራማሪዎችና የሥነምግባር ኮሚቴ በስልክ ማናገር ይችላል፡፡ አለም አብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡0911225832)፣ የአዲስ አበባ ዩኒቨርሲቲ የምርምር ስነምግባር ኮሚቴ (ስልክ፡ 0118961396)

I-D: Inforamtion Sheet (Tigrigna Version)

ትግርኛ ቕዳሕ

ኣርእስቲ ምርምር ፅንዓት፡ 'ፌኖታይፒክ ኤንድ ሞለክዩላር ካራክተራይዜሽን ኦፍ ስታፊሎኮከስ ኦውሪየስ ፍሮም ህዩማን ኤንድ ኣኒማልስ ኢን መቐለ፣ ኖርዘርን ኢትዮጵያ'

ሽም ዋና ተመራማሪ፡ ኣለም ኣብርሃ (ብሜዲካል ማይክሮባዮሎጂ ተምሃራይ ሳልሳይ ዲግሪ)

ሽም ድርጅት፡ ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኮሌጅ ጥዕና ሳይንስ ፣ ክፍሊ ት/ቲ ማይክሮባዮሎጂ፣ ኢሙኖሎጂን ፓራሲቶሎጂን

ዋና ስፖንሰር፡ ዩኒቨርሲቲ ኣዲስ ኣበባ ፣ ኦሃዮ ስቴት ዩኒቨርሲቲ

መእተዊ፡ ስታፊሎኮከስ ኦውሪየስ ኣብ ዝተፈላለዩ ኣካላት ሰዉነትና ዝርከብን ኣብ ኩሉ ክፍሊ ኣካላት ሰዉነትና ኢንፌክሽን ከምፅእ ዝኽእል ባክተርያ እዩ፡፡ ስታፊሎኮከስ ኦውሪየስ ኢንፌክሽን ቆርበትን ድሕሪ-መጥባሕቲ ቑስልን ብምምፃእ ቀዳማይ እዩ፡፡ እዚ ባክተርያ ካብ ሰብ ወፃኢ ኣብ ዝተፈላለዩ እንስሳታት ኢንፌክሽን ዘምፅእ እንትኾን፤ ኣብ ናይ ፀባ ከፍቲ ኢነፍክሽን ጡብ (ማስታይተስ) ብምምፃእ ይፍለጥ፡፡ ብስታፊሎኮከስ ኦውሪየስ ዝተጠቕዑ እንስሳት እቲ ባክተርያ ናብ ሰብ ምምሕልላፍ ከምዝኽእል ካብ ሃገርና ወፃኢ ዝተሰርሑ ምርምር ፅንዓታት የረድኡ፡፡ ስታፊሎኮከስ ኦውሪየስ ኣብ ሰብን እንስሳታትን ዘምፅኦ ሳዕቤን ጥዕና ዓብዪኳ እንተኾነ ኣብ ሃገርና ኣብዚ ባክተርያ ዘሎ ብምርምር ዝተደገፈ ሓበሬታ ውስን እዩ፡፡ ብፍላይ ኣብ ሃገርና ዘለዉ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ስርጭቶምን፣ እዚ ባከተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት፣ እዚ ባክተርያ ዝተሸከሞም ኩነታት መርዛማ ነገራት (ቶክሲንስ) ምንም ዓይነት መረዳእታ የለን፡፡

ተሳተፍቲ ምርምር ፅንዓት፡ እዚ ምርምር ፅንዓት ኣብ ሕብረተሰብ ዉሽጢ ዝተኸሰተ ናይ ቆርበት ኢንፌክሽን ዘለዎም ፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊ ዘለዎም፣ ኣብ ሕርሻ ፍርያት ፀባ ዝሰርሑ ሰባትን ኣብኡ ዝርከባ ኣላሕምን ዘካትት እዩ፡፡ ስለዚ ኣብዚ ምርምራዊ መፅናዕቲ ንክትሳተፍ/ፊ ንጋብዝ ኣለና፡፡

ሽቶ እቲ ምርምር ፅንዓት፡ ብስታፊሎኮከስ ኦውሪየስ ዝመፅእ ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባ ንምፍላጥ፤ ነዞም ዝተጠቐሱ ሕማማት ዘምፀኡ ዓይነታት ስታፊሎኮከስ ኦውሪየስን ሥርጭቶምን ንምርዳእ፤ ከምኡዉን እዚ ባክተርያ ኣብ መንጎ ሰብን እንስሳን ዘለዎ ናይ ምትሕልላፍ ኩነታት ንምፍላጥ እዩ፡፡

እቲ ምርምር ፅንዓት ዝወስዶ ግዘ፡ ክሳብ ኣርባዕተ ኣዋርሕ ክወስድ ተባሂሉ ይግመት፡፡ ይኹን እምበር ካባኻ/ኺ ዝዉሰድ ናይ ላቦራቶሪ ናሙና ሓደ ግዘ ጥራሕ እዩ፡፡

ቅደም ተከተል ኣተገባብራ እቲ ፅንዓት፡ መጀመርያ ነቲ ፅንዓት ዝምልከቱ ውሑዳት ሕቶታት ክንጥይቐካ/ኪ ኢና፡፡ ብምቕፃል ካብቲ እንፌክሽን ዘለዎ ናይ ቖርበካ/ኪ ክፍሊ ንላቦራቶሪ ምርመራ ዝውዕል ናሙና (መግሊ) ብዝሰልጠነ/ት ነርስ ይዉሰድ፡፡ እቲ ናሙና ንምርመራ ናብ ላቦራቶሪ ይዉሰድሞ ስታፊሎኮከስ ኦውሪየስ እንተተረኺቡ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ይኸይድ፡፡

ኣብቲ ምርምር ፅንዓት እንትሳተፉ ከጋጥም ዝኽእል ፀገም፡ ናይ ላቦራቶሪ ናሙና ኣብ ዝዉሰደሉ እዋን ብጣዕሚ ንኡሽተይ ሕማም ክስመዐካ/ኪ ይኽእል እዩ፡፡ ነዙይ ንምቕራፍ እቲ ናሙና ብዝሰልጠነ/ት ነርስ ብጥንቃቐ ክዉሰድ እዩ፡፡

ረብሓ፡ ኹሉ ናይ ላቦራቶሪ ምርመራ ብነፃ ዝግበር እንትኸዉን ዉፅኢት ናይቲ ምርመራ ዝሓሸ ሕክምና መአንታን ክትረክብ/ቢ ንዝከታተለካ/ኪ ሓኪም ይወሃብ፡፡ ኣብዚ ምርምር ፅንዓት ብምስታፎኻ/ኺ ካባኻ/ኽን ካብ ካልኦትን እንረኽቦ መረዳእታ ብስታፊሎኮከስ ኦውሪየስ ምኽንያት ዝመፅእ ናይ ቆርበት ኢንፌክሽን መከላኸልን መቆፃፀርን ሜላ ንምንዳፍ ዓብዪ ረብሓ አለዎ፡፡

ሚስጢራዊነት፡ ክብርን ሚስጢርን ተሳተፍቲ ናይዚ ፅንዓት ብዘተኣማምን ከምንሕሉ ክነረጋግፅ ንፎቱ፡፡ መንነትኻ/ኺ ዝሕብር ምንም ዓይነት መረዳእታ ካብቲ ናይ ምርምር ጉጅለ ወፃኢ ንማንም ኣይነካፍልን፡፡ እቲ መፅናዕቲ እብ ዝሕተመሉ እዋን ሽምካ/ኪ ኣይፀሓፍን፡፡ ካባኻ/ኺ እንረኽቦ መረዳእታ ኣብ ዝቑለፍ ሳንዱቕ ብጥንቃቐ ዝቕመጥ እንትኸዉን ኣብ ኮምፒውተር ዝቕመጥ መረዳእታ ድማ ብፓስዎርድ ይቑለፍ፡፡

ናይ ምስታፍ ፈቓደኝነት፡ ኣብዚ ፅንዓት ምስታፍ ሙሉእ ብሙሉእ ኣብ ፈቓድካ/ኪ ዝተመስረተ እንትኸዉን ናይ ዘይምስታፍ መሰልኻ/ኺ ዝተሓለወ እዩ፡፡ ዋላ ድሕሪ ፍቓድ ምሃብካ/ኪ ኣብዝኾነ ግዘ ካብቲ መፅናዕቲ ክትግል/ሊ ተኽእል/ሊ ኢኻ/ኺ፡፡ እዚ ዉሳነ ንሐዚ ወይ ድማ ንቐፃሊ ኣብዚ ጥዕና ተቋም ንትረኽቦ/ብዮ ኣገልግሎት ሕክምና ዝፈጥሮ ፀገም ኣይህልን፡፡

አድራሻ፡ ኣብዚ ፅንዓት ሕቶ እንተልይካ/ኪ ሐዚ ምጥያቕ ትኽእል/ሊ እንትኾን/ኒ ተወሳኺ መብራህርሂ እንተደሊኻ/ኺ ድማ ነዞም ዝቕፅሉ ተመራመርትን ስነምግባር ምርምር ኮሚቴን ምርካብ ይከኣል፡፡ አለም አብርሃ (ስልክ፡ 0911784037)፣ ዶ/ር ዳንኤል አስራት (ስልክ፡ 0911223019)፣ ዶ/ር ይምጡበዝናሽ ወ/አማኑኤል (ስልክ፡ 0911225832)፣ ናይ አዲስ አበባ ዩኒቨርሲቲ ኮሚቴ ስነምግባር ምርምር (ስልክ 0118961396)

Annex II: Concent forms

Annex II-A: Consent form for study participants with skin and soft tissue infections

II-A: Consent form English Version

I have been requested to participate in this study which involves collection of wound swab/pus specimen and in which I will answer few questions. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about burden of *Staphylococcus aureus* in hospitals and dairy farms found in Mekele ; which is one of the leading causes of community acquired skin and soft tissue infections, post-operative wound infection and bovine mastitis in the world. I have also informed that the bacteria will be transported abroad for further investigation with appropriate handling and for the study purpose only. Furthermore, I have informed that the information obtained from me will be kept confidential and used for the study purpose. I have asked some questions and clarification has been given to me. I have given my consent to participate in the study and I hereby confirm my agreement with my signature.

	Full Name	Signature	Date
Study participant			
Consent taker			
Witness			

II-A: Consent form Amharic Version

አማርኛ ቅጅ

በዚህ የምርምር ጥናት በመሳተፍ የላቦራቶሪ ናሙና እንድሰጥና ጥቂት ጥያቄዎችን እንድመልስ ተጋብዣለሁ፡፡ የጥናቱ ዓላማና የላቦራቶሪ ናሙናው አሰባሰብ ተብራርቶልኛል፡፡ የምርምር ጥናት ማብራርያዉን አንብቤዋለሁ (ተነቦልኛል)፣ በመሆኑም የጥናቱ ዓላማ መቐለ በሚገኙ ሆስፒታሎች እና የላም ወተት እርሻዎች በዓለማችን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የወተት ላም ጡት ኢንፌክሽን ከሚያመጡ ጥቃቅን ታህዋስያን ግንባር ቀደም የሆነዉን ስታፊሎኮከስ ኦውሪየስ የተባለ ባክተርያ ለማጥናት እንደሆነ ተረድቻለሁ፡፡ በተጨማሪም ባክተርያዉን ለተጨማሪ ምርመራ በጥንቃቄ ተይዞ ወደ ዉጭ ሃገር እንደሚሄድ ተገልፆልኛል፡፡ እነዲሁም ከኔ የሚገኘዉን ማነኛዉም መረጃ በሚስጢር እነደሚያዝና ለጥናቱ ዓላማ ብቻ እነደሚዉል ተረድቻለሁ፡፡ በስተመጨረሻም ግልፅ ያልሆኑልኝን ጥያቄዎችን ጠይቄ በቂ ማብራርያ አግኝቻለሁ፡፡

በመሆኑም በጥናቱ ለመሳተፍ ፈቃደኛ ነኝ፡፡ ፈቃደኝነቴንም በፊርማዬ አረጋግጣለሁ፡፡

	ሙሉ ሥም	ፊርማ	ቀን
የጥናቱ ተሳታፊ			
ፈቃደኝነቱን የወሰደው ባለሙያ			
ምስክር			

II-A: Consent form Tigrigna Version

<u>ትግርኛ ቕዳሕ</u>

ኣብዚ ናይ ምርምር ፅንዓት ብምስታፍ ናሙና ላቦራቶሪ ንክህብን ዉሑዳት ሕቶታት ንክምልስን ተጋቢዘ ኣለኹ፡፡ ናይቲ ፅንዓት ሽቶን ናሙና ላቦራቶሪ አሰባስባን ተገሊፁለይ እዩ፡፡ ሓበሬታ እቲ ምርምር ፅንዓት ኣንቢበዮ እየ (ተነቢቡለይ እዩ)፡፡ ሽቶ እቲ ምርምር ፅንዓት ኣብ መቐለ ዝርከቡ ሆሰፒታላትን ሕርሻ ፍርያት ኣላሕም ፀባን ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባን ካብ ዘምፅኡ ጀርምታት እቲ ቀዳሚ ስፍራ ዝሕዝ ስታፊሎኮከስ ኦውሪየስ ዝተብሃለ ባክተርያ ንምፍላይ ከምዝኾ ተረዲአ እየ፡፡ ከምኡዉን እቲ ባክተርያ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ከምዝኸይድ ተነጊሩኒ እዩ፡፡ ካባይ ዝርከብ ምንም ዓይነት መረዳእታ ብሚስጥር ከምዝተሓዝን ነቲ ምርምር ፅንዓት ሽቶ ጥራይ ከምዝውዕልን ተገሊፁለይ እዩ፡፡ ኣብ መጨረሻ ዘይበርሁለይ ሕቶታት ጠይቐ ኣዕጋቢ መልሲ ረኺበ እየ፡፡

ስለዚ ኣብቲ ምርምር ፅንዓት ንምሰታፍ ፍቓደኛ እየ፡፡ ፍቓደኝነተይ ድማ ብፊርማይ የረጋግፅ ኣለኹ፡፡

	ሙሉእ ሽም	**ፊርማ**	**ዕለት**
ተሳታፊ እቲ ምርምር ፅንዓት			
ፍቓደኝነት ዝወሰደ በዓልሞያ			
ምስክር			

Annex II-B: Consent form for dairy farm workers

II-B: Consent form English Version

I have been requested to participate in this study which involves collection of specimen and in which I will answer few questions. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about burden of *Staphylococcus aureus* in hospitals and dairy farms found in Mekele ; which is one of the leading causes of community acquired skin and soft tissue infections, post-operative wound infection and bovine mastitis in the world. This bacterium is also found in the nares of healthy individuals. I have also informed that the bacteria will be transported abroad for further investigation with appropriate handling and for the study purpose only. Furthermore, I have informed that the information obtained from me will be kept confidential and used for the study purpose. I have asked some questions and clarification has been given to me. I have given my consent to participate in the study and I hereby confirm my agreement with my signature.

	Full Name	**Signature**	**Date**
Study participant			
Consent taker			
Witness			

II-B: Consent form Amharic Version

<u>አማርኛ ቅጅ</u>

በዚህ የምርምር ጥናት በመሳተፍ የላቦራቶሪ ናሙና እንድሰጥና ጥቂት ጥያቄዎችን እንድመልስ ተጋብዣለሁ፡፡ የጥናቱ ዓላማና የላቦራቶሪ ናሙናው ኣሰባሰብ ተብራርቶልኛል፡፡ የምርምር ጥናት ማብራርያዉን አንብቤዋለሁ (ተነቦልኛል)፣ በመሆኑም የጥናቱ ዓላማ መቐለ በሚገኙ ሆሰፒታሎች እና የላም ወተት እርሻዎች በዓለማችን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የወተት ላም ጡት ኢንፌክሽን ከሚያመጡና በብዙ ሰዎች አፍንጫ ውስጥ ከሚገኙ ጥቃቅን ታህዋስያን ግንባር ቀደም የሆነዉን ስታፊሎኮከስ ኦውሪየስ የተባለ ባክተርያ ለማጥናት እንደሆነ ተረድቻለሁ፡፡ በተጨማሪም ባክተርያዉን ለተጨማሪ ምርመራ በጥንቃቄ ተይዞ ወደ ዉጭ ሃገር እንደሚሄድ ተገልፆልኛል፡፡ እነዲሁም ከኔ የሚገኘዉን ማነኛዉም መረጃ በሚስጢር እነደሚያዝና ለጥናቱ ዓላማ ብቻ እነደሚዉል ተረድቻለሁ፡፡ በስተመጨረሻም ግልፅ ያልሆኑልኝን ጥያቄዎችን ጠይቄ በቂ ማብራርያ አግኝቻለሁ፡፡

በመሆኑም በጥናቱ ለመሳተፍ ፈቃደኛ ነኝ፡፡ ፈቃደኝነቴንም በፊርማዬ አረጋግጣለሁ፡፡

	ሙሉ ሥም	ፊርማ	ቀን
የጥናቱ ተሳታፊ			
ፈቃደኝነቱን የወሰደው ባለሙያ			
ምስክር			

II-B: Consent form Tigrigna Version

<u>ትግርኛ ቅዳሕ</u>

ኣብዚ ናይ ምርምር ፅንዓት ብምስታፍ ናሙና ላቦራቶሪ ንክህብን ዉሑዳት ሕቶታት ንክምልስን ተጋቢዘ ኣለኹ፡፡ ናይቲ ፅንዓት ሽቶን ናሙና ላቦራቶሪ አሰባስባን ተገሊፁለይ እዩ፡፡ ሓበሬታ እቲ ምርምር ፅንዓት ኣንቢበዮ እየ (ተነቢቡለይ እዩ)፡፡ ሽቶ እቲ ምርምር ፅንዓት ኣብ መቐለ ዝርከቡ ሆስፒታላትን ሕርሻ ፍርያት ኣላሕም ፀባን ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባን ካብ ዘምፅኡን ኣብ ኣፍንጫ ብዙሓት ስባት ካብ ዝርከቡ ጀርምታት እቲ ቀዳማይ ስፍራ ዝሕዝ ስታፊሎኮከስ ኦውሪየስ ዝተብሃለ ባክተርያ ንምፍላይ ከምዝኾ ተረዲአ እየ፡፡ ከምኡዉን እቲ ባክተርያ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ከምዝኸይድ ተነጊሩኒ እዩ፡፡ ካባይ ዝርከብ ዝኾነ ዓይነት መረዳእታ ብሚስጥር ከምዝትሓዝን ነቲ ምርምር ፅንዓት ሽቶ ጥራይ ከምዝውዕልን ተገሊፁለይ እዩ፡፡ ኣብ መጨረሻ ዘይበርሁለይ ሕቶታት ጠይቐ ኣዕጋቢ መልሲ ረኺበ እየ፡፡

ስለዚ ኣብቲ ምርምር ፅንዓት ንምሰታፍ ፍቓደኛ እየ፡፡ ፍቓደኝነተይ ድማ ብፊርማይ የረጋግፅ ኣለኹ፡፡

	ሙሉእ ሽም	ፊርማ	ዕለት
ተሳታፊ እቲ ምርምር ፅንዓት			
ፍቓደኝነት ዝወሰደ በዓልሞያ			
ምስክር			

Annex II-C: Consent form for Parents/guardians

II-C: Consent form English Version

I have been requested to let my child participate in this study which involves collection of specimen and in which I will answer few questions. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about burden of *Staphylococcus aureus* in hospitals and dairy farms found in Mekele ; which is one of the leading causes of community acquired skin and soft tissue infections, post-operative wound infection and bovine mastitis in the world. I have also informed that the bacteria will be transported abroad for further investigation with appropriate handling and for the study purpose only. Furthermore, I have informed that the information obtained from my child will be kept confidential and used for the study purpose. I have asked some questions and clarification has been given to me. I have given my consent to let my child participate in the study and I hereby confirm my agreement with my signature.

	Full Name	**Signature**	**Date**
Parent/guardian			
Consent taker			
Witness			

II-C: Consent form Amharic Version

<u>**አማርኛ ቅጅ**</u>

በዚህ የምርምር ጥናት ልጄ እንዲሳተፍ/እንድትሳተፍ እና የላቦራቶሪ ናሙና እንዲሰጥ/እንድትሰጥ እንዲሁም ጥቂት ጥያቄዎችን እንድመልስ ተጋብዣለሁ፡፡ የጥናቱ ዓላማና የላቦራቶሪ ናሙናው ኣሰባሰብ ተብራርቶልኛል፡፡ የምርምር ጥናት ማብራርያዉን አንብቤዋለሁ (ተነቦልኛል)፣ በመሆኑም የጥናቱ ዓላማ መቐለ በሚገኙ ሆሰፒታሎች እና የላም ወተት እርሻዎች በዓለማችን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የወተት ላም ጡት ኢንፌክሽን ከሚያመጡ ጥቃቅን ታህዋስያን ግንባር ቀደም የሆነዉን ስታፊሎኮከስ ኦውሪየስ የተባለ ባክተርያ ለማጥናት እንደሆነ ተረድቻለሁ፡፡ በተጨማሪም ባክተርያዉን ለተጨማሪ ምርመራ በጥንቃቄ ተይዞ ወደ ዉጭ ሃገር እንደሚሄድ ተገልፆልኛል፡፡ እነዲሁም ከልጄ የሚገኘዉን ማነኛዉም መረጃ በሚስጢር እነደሚያዝና ለጥናቱ ዓላማ ብቻ እነደሚዉል ተረድቻለሁ፡፡ በስተመጨረሻም ግልፅ ያልሆኑልኝን ጥያቄዎችን ጠይቄ በቂ ማብራርያ አግኝቻለሁ፡፡

በመሆኑም ልጄ በጥናቱ እንዲሳተፍ/እንድትሳተፍ ፈቃደኛ ነኝ፡፡ ፈቃደኝነቴንም በፊርማዬ አረጋግጣለሁ፡፡

	ሙሉ ሥም	ፊርማ	ቀን
ወላጅ/ተንከባካቢ			
ፈቃደኝነቱን የወሰደው ባለሙያ			
ምስክር			

II-C: Consent form Tigrigna Version

<u>**ትግርኛ ቕዳሕ**</u>

ኣብዚ ናይ ምርምር ፅንዓት ቀልዐአይ ክሳተፍ/ክትሳተፍን ናሙና ላቦራቶሪ ክህብ/ክትህብ ክፈቅድን ከምኡዉን ዉሑዳት ሕቶታት ንክምልስን ተጋቢዘ ኣለኹ፡፡ ናይቲ ፅንዓት ሽቶን ናሙና ላቦራቶሪ አሰባስባን ተገሊፁለይ እዩ፡፡ ሓበሬታ እቲ ምርምር ፅንዓት ኣንቢበዮ እየ (ተነቢቡለይ እዩ)፡፡ ሽቶ እቲ ምርምር ፅንዓት ኣብ መቐለ ዝርከቡ ሆሰፒታላትን ሕርሻ ፍርያት ኣላሕም ፀባን ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባን ካብ ዘምፅኡ ጀርምታት እቲ ቀዳሚ ስፍራ ዝሕዝ ስታፊሎኮከስ ኦውሪየስ ዝተብሃለ ባክተርያ ንምፍላይ ከምዝኾ ተረዲአ እየ፡፡ ከምኡዉን እቲ ባክተርያ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ከምዝኸይድ ተነጊሩኒ እዩ፡፡ ካብ ቆልዓይ ዝርከብ ምንም ዓይነት መረዳእታ ብሚስጥር ከምዝተሓዝን ነቲ ምርምር ፅንዓት ሽቶ ጥራይ ከምዝውዕልን ተገሊፁለይ እዩ፡፡ ኣብ መጭጨረሻ ዘይበርሁለይ ሕቶታት ጠይቐ ኣፅጋቢ መልሲ ረኺበ እየ፡፡

ስለዚ ኣብቲ ምርምር ፅንዓት ቆልዓይ ንክሳተፍ/ንክትሳተፍ ፍቓደኛ እየ፡፡ ፍቓደኝነተይ ድማ ብፊርማይ የረጋግፅ ኣለኹ፡፡

	ሙሉእ ሽም	ፊርማ	ዕለት
ወላዲ/ተንከባኻቢ			
ፍቓደኝነት ዝወሰደ በዓልሞያ			
ምስክር			

Annex II-D: Assent form for 12-18 years old study participants with soft tissue infections

II-D: Consent form English Version

I have been requested to participate in this study which involves collection of wound swab/pus specimen and in which I will answer few questions. The purpose of the study and sample collection procedure has been explained to me. I have also read the information sheet (or it has been read to me); I have understood that this study is about burden of *Staphylococcus aureus* in hospitals and dairy farms found in Mekele ; which is one of the leading causes of community acquired skin and soft tissue infections, post-operative wound infection and bovine mastitis in the world. I have also informed that the bacteria will be transported abroad for further investigation with appropriate handling and for the study purpose only. Furthermore, I have informed that the information obtained from me will be kept confidential and used for the study purpose. I have asked some questions and clarification has been given to me. I have given my consent to participate in the study and I hereby confirm my agreement with my signature.

	Full Name	**Signature**	**Date**
Study participant			
Assent taker			
Witness			

II-D: Consent form Amharic Version

አማርኛ ቅጅ

በዚህ የምርምር ጥናት በመሳተፍ የላቦራቶሪ ናሙና እንድሰጥና ጥቂት ጥያቄዎችን እንድመልስ ተጋብዣለሁ፡፡ የጥናቱ ዓላማና የላቦራቶሪ ናሙናው ኣሰባሰብ ተብራርቶልኛል፡፡ የምርምር ጥናት ማብራርያዉን አንብቤዋለሁ (ተነቦልኛል)፣ በመሆኑም የጥናቱ ዓላማ መቐለ በሚገኙ ሆስፒታሎች እና የላም ወተት እርሻዎች በዓለማችን የቆዳ ኢንፌክሽን፣ የድህረ ቀዶ ጥገና ቁስል ኢንፌክሽን እና የወተት ላም ጡት ኢንፌክሽን ከሚያመጡ ጥቃቅን ታህዋስያን ግንባር ቀደም የሆነዉን ስታፊሎኮከስ ኦውሪየስ የተባለ ባክተርያ ለማጥናት እንደሆነ ተረድቻለሁ፡፡ በተጨማሪም ባክተርያዉን ለተጨማሪ ምርመራ በጥንቃቄ ተይዞ ወደ ዉጭ ሃገር እንደሚሄድ ተገልፆልኛል፡፡ እነዲሁም ከኔ የሚገኘዉን ማነኛዉም መረጃ በሚስጢር እነደሚያዝና ለጥናቱ ዓላማ ብቻ እነደሚዉል ተረድቻለሁ፡፡ በስተመጨረሻም ግልፅ ያልሆኑልኝን ጥያቄዎችን ጠይቄ በቂ ማብራርያ አግኝቻለሁ፡፡

በመሆኑም በጥናቱ ለመሳተፍ ፈቃደኛ ነኝ፡፡ ፈቃደኝነቴንም በፊርማዬ አረጋግጣለሁ፡፡

	ሙሉ ሥም	ፊርማ	ቀን
የጥናቱ ተሳታፊ			
ፈቃደኝነቱን የወሰደው ባለሙያ			
ምስክር			

II-D: Consent form Tigrigna Version

<u>ትግርኛ ቕዳሕ</u>

ኣብዚ ናይ ምርምር ፅንዓት ብምስታፍ ናሙና ላቦራቶሪ ንክህብን ዉሑዳት ሕቶታት ንክምልስን ተጋቢዘ ኣለኹ፡፡ ናይቲ ፅንዓት ሽቶን ናሙና ላቦራቶሪ አሰባስባን ተገሊፁለይ እዩ፡፡ ሓበሬታ እቲ ምርምር ፅንዓት ኣንቢበዮ እየ (ተነቢቡለይ እዩ)፡፡ ሽቶ እቲ ምርምር ፅንዓት ኣብ መቐለ ዝርከቡ ሆሰፒታላትን ሕርሻ ፍርያት ኣላሕም ፀባን ናይ ቆርበት ኢንፌክሽን፣ ኢንፌክሽን ድሕሪ-መጥባሕቲ ቑስሊን እንፌክሽን ጡብ ኣላሕም ፀባን ካብ ዘምፅኡ ጀርምታት እቲ ቀዳሚ ስፍራ ዝሕዝ ስታፊሎኮከስ ኦውሪየስ ዝተብሃለ ባክተርያ ንምፍላይ ከምዝኾ ተረዲአ እየ፡፡ ከምኡዉን እቲ ባክተርያ ንተወሳኺ ምርመራ ናብ ወፃኢ ዓዲ ከምዝኸይድ ተነጊሩኒ እዩ፡፡ ካባይ ዝርከብ ምንም ዓይነት መረዳእታ ብሚስጥር ከምዝተሓዝን ነቲ ምርምር ፅንዓት ሽቶ ጥራይ ከምዝውዕልን ተገሊፁለይ እዩ፡፡ ኣብ መጨረሻ ዘይበርሁለይ ሕቶታት ጠይቐ ኣዕጋቢ መልሲ ረኺበ እየ፡፡

ስለዚ ኣብቲ ምርምር ፅንዓት ንምሰታፍ ፍቓደኛ እየ፡፡ ፍቓደኝነተይ ድማ ብፊርማይ የረጋግፅ ኣለኹ፡፡

	ሙሉእ ሽም	ፊርማ	ዕለት
ተሳታፊ እቲ ምርምር ፅንዓት			
ፍቓደኝነት ዝወሰደ በዓልሞያ			
ምስክር			

Annex III: Questionnaire/ Data collection forms

Annex III-A: Data collection sheet for study participants with community onset Skin and soft tissue infections

Annex III-A: English Version

Code: ………..

Name of Data collector: …………………… Date: ________/_______/_________

Q1	**Name of health institution**	**1. Ayder referral hospital** **2. Other, specify …………………..**
Q2	Department	1. Emergency 2. Surgery 3. Pediatrics OPD 4. Adult medical OPD 5. Other, specify ________________________________
Q3	Patient card number	………………..
Q4	Residence	1. Rural 2. Urban
Q5	Patient address	Region………………… Zone ………………. Woreda …………………. Kebelle …………………
Q6	Date of Hospital visit	____/____/______ E.C
Q7	Sex	1. Male 2. Female
Q8	Age in years	_______ years
Q9	Occupation	……………………
Q10	Have you taken any antibiotics in the past one year?	1. Yes, specify the drug name? ……………………… 2. No
Q11	Have you had surgery in the past one year?	1. Yes, specify the site of surgery? ……………………… 2. No
Q12	How often do you visit health care/hospital in the past one year?	………………………………..
Q13	Have you had SSTI in the past 1 year?	1. Yes, specify site of infection? ………………………… 2. No
Q14	Is there any household member with SSTIs currently?	1. Yes, specify your relation with the member? ……… 2. No
Q15	Was there any household member with SSTIs in the past 1 year?	1. Yes, specify your relation with the member? ……… 2. No
Q16	Do you have any domestic animals?	1. Yes, list them? ………………………………………… 2. No
Q17	Type of SSTI	1. Impetigo 2. Boil 3. Folliculitis 4. Carbuncle 5. Other, specify …………………………….
Q18	Location of SSTIs	1. Abdomen 2. Back 3. Thorax 4. Head and neck 5. Leg 6. Other, Specify_______
Q19	Prescribed antibiotic(s) at time of acute SSTI	…………………………………………………………

Annex III-B: Data collection sheet for patients with clinically suspected surgical site infections

Annex III-B: English Version

Code: ……………

Name of Data collector: __________________ Date: ________/_______/_________

1.	**Hospital Name**	**1. Ayder referral hospital** **2. Mekele hospital**
2.	Hospital department	1. Surgical 2. Gynecology and obstetrics 3. Other, specify ……………………………...
3.	Patient ID	………………………
4.	Date of Hospital admission	________/_______/_________
5.	Sex	1. Male 2. Female
6.	Age at date of operation in years	……… years
7.	Residence	1. Rural 2. Urban
8.	Residence address	Region………………… Zone ………………. Woreda …………………. Kebelle …………………
9.	Occupation	…………………..
10.	Date of Operation	________/_______/_________
11.	Operation ID	………………………………..
12.	Was an endoscope used during the procedure?	1. Yes 2. No
13.	Wound contamination class	1. Clean 2. Contaminated 2. Clean-contaminated 4. Dirty or infected
14.	Location of wound	1. Abdomen 2. Back 3. Thorax 4. Head and neck 5. Leg 6. Other, Specify…………….…
15.	Duration of operation in minutes (from skin incision to skin closure)	……… to ………
16.	Was antibiotic prophylaxis given?	1. Yes, list the antibiotics used………………..………. 2. No
17.	Has the patient developed a surgical site infection?	1. Yes 2. No → **Go to Nº 2o**
18.	If yes, specify date of onset of SSI	_____/_____/________
19.	Type of SSI	1. Superficial incisional 2. Deep incisional 2. Organ/space 4. Unknown
20.	Hospital stay	From ___/_____/___ to ___/_____/___
21.	Outcome of the patient	1. Discharge 2. Transfer to other hospital 3. Death 4. Other, specify …………………………..
22.	**Date of outcome**	_____/_____/______

Annex III-C: Data collection sheet for dairy farm workers in and around Mekele

Annex III-C: English Version

Code: ………..

Name of Data and sample collector: ………………… Date: _____/_____/_____ E.C

1	**Dairy farm name**	**…………………..**
2	Location	1. Rural 2. Urban
3	Address	Region………………… Zone ………………. Woreda …………………. Kebelle …………………
4	Sex	1. Male 2. Female
5	Age in years	_______ years
6	Specific duty in the farm	…………………..
7	For how long do you serve in this farm?	_______ years
8	Have you ever worked in a dairy farm before joing here?	1. YES, for how long ……. years 2. NO
9	Do you work in other dairy farm other than this dairy farm now?	1. YES, specify name of the dairy farm 2. NO

Annex III-D: Data collection sheet for lactating dairy cows in Mekele

III-D: English Version

Code: ………..

Name of Data and sample collector: …………………… Date: ______/______/_____E.C

<table>
<tr><td>1</td><td colspan="3">Dairy farm name</td><td colspan="2">…………………..</td></tr>
<tr><td>2</td><td colspan="3">Age in years</td><td colspan="2">_______ years</td></tr>
<tr><td>3</td><td colspan="3">Location</td><td colspan="2">1. Rural 2. Urban</td></tr>
<tr><td>4</td><td colspan="3">Breed type</td><td colspan="2">1. Local breed
2. Other, specify ___________________</td></tr>
<tr><td>5</td><td colspan="3">How long is after calving? (in days)</td><td colspan="2">……….. days</td></tr>
<tr><td>6</td><td colspan="3">Parity (How many times does this cow give birth?)</td><td colspan="2">……………</td></tr>
<tr><td>7</td><td colspan="3">Did this cow take any antibiotics within the last one year?</td><td colspan="2">1. Yes, specify the drug/s ………………………………………..
2. No</td></tr>
<tr><td>8</td><td colspan="3">Milking process</td><td colspan="2">1. Manual (using hands)
2. Using machine</td></tr>
<tr><td colspan="2" rowspan="2">Visual inspection of udder quarters</td><td>9</td><td colspan="2">Teat lesion:</td><td>1. Yes, specify udder quarter………
2. No</td></tr>
<tr><td>10</td><td colspan="2">Udder Swelling</td><td>1. Yes, specify udder quarter ……….
2. No</td></tr>
<tr><td colspan="2" rowspan="3">Visual inspection of milk</td><td>11</td><td colspan="2">Viscosity</td><td>1. Normal
2. Watery, specify udder quarter…….
3. Thicker than normal, specify udder quarter…</td></tr>
<tr><td>12</td><td colspan="2">Color</td><td>1. Normal
2. Yellow, specify udder quarter…….
3. Blood-tinged, specify udder quarter……</td></tr>
<tr><td>13</td><td colspan="2">Consistency</td><td>1. Normal
2. 13Flakes or clots; specify udder quarter……</td></tr>
</table>

Annex IV: Culture and antimicrobial susceptibility data

1. Culture result

B-hemolysis on Blood agar	Mannitol fermentation	Catalase	Slide coagulase	Tube coagulase	DNase test	***Is the isolate S. aureus?***

2. Antimicrobial susceptibility testing

Antimicrobial disc	Interpretive Criteria (nearest whole mm)			AST result (mm)	Interpretation
	Sensitive	**Intermediate**	**Resistant**		
Penicillin (10 units)	≥29	–	≤28		
Cefoxitin *(30 μg)	≥ 22	–	≤ 21 mm		
Erythromycin (15 μg)	≥23	14-22	≤13		
Clindamycin (2 μg)	≥21	15–20	≤14		
D-test					
Tetracycline (30 μg)	≥19	15–18	≤14		
Trimethoprim-Sulphamethoxazole (1.25/23.75 μg)	≥16	11–15	≤10		
Rifampin (5 μg)	≥20	17–19	≤16		
Vancomycin E-test	≥16 μg/ml	4-8 μg/ml	≤2 μg/ml	_____μg/ml	
Daptomycin E-test	–	–	≤1 μg/ml	_____μg/ml	

* Cefoxitin is used for preliminary screening of MRSA

Annex V: Molecular Characterizations Procedures and Data collection forms

Annex V-A: *nuc* gene detection

PCR mix preparation

	Components	Volume/reaction
1	*nuc* F	1 µl
2	*nuc* R	1 µl
3	Molecular grade water	22 µl
4	DNA template	1 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	94 ^{0}C	2 min
Step 2: 30 cycles	94 ^{0}C	2 min
	60 ^{0}C	1 min
	72 ^{0}C	2 min
Final extension	72 ^{0}C	2 min
Hold at	4 ^{0}C	

Electrophoresis (5 µl of PCR product)

- 1.5% Agarose gel: at 90**V** for 1 hour

S. No	Sample code	Result
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		

S. No	Sample code	Result
17.		
18.		
19.		
20.		
21.		
22.		
23.		
24.		
25.		
26.		
27.		
28.		
29.		
30.		
31.		
32.		

S. No	Sample code	Result
33.		
34.		
35.		
36.		
37.		
38.		
39.		
40.		
41.		
42.		
43.		
44.		
45.		
46.		
47.		
48.		

Annex V-B: Multiplex PCR for detection of *spa*/*pvl*/*mec*A/*mec*C

Forward primers: spa-1113F, pvl-F, mecA P4, $mecA_{LGA251}$ MultiFP
Reverse primers: spa-1514R, pvl-R, mecA P7, $mecA_{LGA251}$ MultiRP
PCR beads: Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA)
PCR products: spa(variable:200-600bp); mecA (162 bp); mecC (138bp); pvl (~85bp)

PCR mix preparation

	Components	Volume/reaction
1	Forward primers	2 µl (0.5µl each)
2	Reverse primers	2 µl (0.5µl each)
3	Molecular grade water	19 µl
4	DNA template	2 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	94 ^{0}C	5 min
Step 2: 30 cycles	94 ^{0}C	30 sec
	59 ^{0}C	1 min
	72 ^{0}C	1 min
Final extension	72 ^{0}C	10 min
Hold at	4 ^{0}C	

Electrophoresis (8 µl of PCR product)

- 2% Agarose gel: at 100**V** for 100 minutes

S. No	Sample code	Result			
		spa	*pvl*	*mec*A	*mec*C
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

Annex V-C: Multiplex PCR for the detection of *sea/seb/sec/seh/sej* genes

Forward primers: *sea-F, seb-F, sec-F, seh-F, sej-F*
Reverse primers: *sea-R, seb-R, sec-R, seh-R, sej-R*
PCR beads: Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA)
PCR products: sea (521bp); seb (667bp); sec (284bp); seh (359bp), sej (142bp)

PCR mix preparation

	Components	Volume/reaction
1	Forward primers	4.25 µl (sea,seb, sej = 0.75µl each; sec, she = 1 µl each)
2	Reverse primers	4.25 µl (sea,seb, sej = 0.75µl each; sec, she = 1 µl each)
3	Molecular grade water	12.5 µl
4	DNA template	4 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	95 ^{0}C	10 min
Step 2: 15 cycles	95 ^{0}C	1 min
	66 ^{0}C	45 secs
	72 ^{0}C	1 min
Step 3: 20 cycles	95 ^{0}C	1 min
	63 ^{0}C	45 secs
	72 ^{0}C	1 min
Final extension	72 ^{0}C	10 min
Hold at	4 ^{0}C	

Electrophoresis (8 µl of PCR product)

- 2% Agarose gel: at 100V for 100 minutes

S. No	Sample code	Result				
		sea	*seb*	*sec*	*seh*	*sej*
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Annex V-D: Multiplex PCR for the detection of *sed/see/seg/sei/tsst-1* genes

Forward primers: sed-F, see-F, seg-F, sei-F, tsst-F
Reverse primers: sed-R, see-R, seg-R, sei-R, tsst-R
PCR beads: Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA)
PCR products: sed (385bp); see (171bp); seg (328bp); sei (466bp), tsst (559bp)

PCR mix preparation

	Components	Volume/reaction
1	Forward primers	4µl (sed,see, seg, sei = 0.75µl each; tsst = 1 µl)
2	Reverse primers	4µl (sed,see, seg, sei = 0.75µl each; tsst = 1 µl)
3	Molecular grade water	13 µl
4	DNA template	4 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	95 ^{0}C	10 min
Step 2: 15 cycles	95 ^{0}C	1 min
	66 ^{0}C	45 secs
	72 ^{0}C	1 min
Step 3: 20 cycles	95 ^{0}C	1 min
	63 ^{0}C	45 secs
	72 ^{0}C	1 min
Final extension	72 ^{0}C	10 min
Hold at	4 ^{0}C	

Electrophoresis (8 µl of PCR product)

- 2% Agarose gel: at 100**V** for 100 minutes

S. No	Sample code	Result				
		sed	*see*	*seg*	*sei*	*tsst*
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Annex V-E: Multiplex PCR for *ccr* typing

Primers: mA1, mA2, α1, α 2, α 3, βc, α4.2, β4.2, γR, γF		
PCR beads: Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA)		
PCR products		
mecA (mA1- mA2)	286	
ccrA1-ccrB (α1- βc) = type 1 ccr	695	
ccrA2-ccrB (α2- βc) = type 2 ccr	937	
crA3-ccrB (α2- βc) = type 3 ccr	1,791	
ccrA4-ccrB4 (α4.2- β4.2) = type 4 ccr	1,287	
ccrC (γR - γF) = type 5 ccr	518	
Remarks: 2 µl of the DNA template. Run: 2% agarose gel run at 130V for 1h		

PCR mix preparation

	Components	Volume/reaction
2	Primers	5 µl (0.5µl each)
3	Molecular grade water	19 µl
4	DNA template	1 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	94 ^{0}C	2 min
Step 2: 30 cycles	94 ^{0}C	2 min
	57 ^{0}C	1 min
	72 ^{0}C	2 min
Final extension	72 ^{0}C	2 min
Hold at	4 ^{0}C	

Electrophoresis (8 µl of PCR product)

- 2% Agarose gel: at 90**V** for 1:30 hours

S. No	Sample code	Ccr type				
		ccr1	*ccr2*	*ccr3*	*ccr4*	*ccr5*
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Annex V-F: Multiplex PCR for detection of *mec*A class and SCC*mec* typing

Primers: mI6, IS7, IS2(iS-2), mA7		
PCR beads: Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA)		
PCR products		
mecA-*mecI* (mA7- mI6) = class A	1,963	
mecA-IS*1272* (mA7-IS7) = class B	2,827	
mecA-IS*431* (mA7-IS2 [iS-2]) = class C	804	
Remarks: 2 µl of the DNA template. Run: 2% agarose gel run at 130V for 1h		

PCR mix preparation

	Components	Volume/reaction
2	Primers	2 µl (0.5µl each)
3	Molecular grade water	22 µl
4	DNA template	1 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	94 ^{0}C	2 min
Step 2: 30 cycles	94 ^{0}C	2 min
	60 ^{0}C	1 min
	72 ^{0}C	2 min
Final extension	72 ^{0}C	2 min
Hold at	4 ^{0}C	

Electrophoresis (8 µl of PCR product)

- 2% Agarose gel: at 90**V** for 1:30 hours

S. No	Sample code	*mec*A class			*ccr* type	SCC*mec* type
		A	B	C		
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Annex V-G: Multiplex PCR setup and data collection form for SCC*mec* IV sub-typing

Primers: 1a3, 1a4, 4al, 4a3, 4b3, 4b4, 4c4, 4c5, 4d3, d4		
PCR beads: Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare Bio-Sciences, USA)		
PCR products		
E007 in type I.1 SCC*mec* (1a3-la4)	154 bp	
CQ02 in type IV.1 (IVa) SCC*mec* (4al-4a3)	458 bp	
M001 in type IV.2 (IVb) SCC*mec* (4b3-4b4)	726 bp	
CR008 in type IV.3 (IVc) SCC*mec* (4c4-4c5)	259 bp	
D002 in type IV.4 (IVd) SCC*mec* (4d3-4d4)	1,242 bp	
Remarks: 2 µl of the DNA template. Run: 2% agarose gel run at 130V for 1h		

PCR mix preparation

	Components	Volume/reaction
2	Primers	5 µl (0.5µl each)
3	Molecular grade water	19 µl
4	DNA template	1 µl
	Total	25 µl

Thermo-cycler setup

Steps	Temp.	Time
Step 1: initial denaturation	94 ^{0}C	2 min
Step 2: 30 cycles	94 ^{0}C	2 min
	60 ^{0}C	1 min
	72 ^{0}C	2 min
Final extension	72 ^{0}C	2 min
Hold at	4 ^{0}C	

Electrophoresis (8 µl of PCR product)

- 2% Agarose gel: at 90**V** for 1:30 hours

S. No	Sample code	*SCCmec IV sub-type*			
		IVa	IVb	IVc	IVd
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

Annex VI: Laboratory procedures and Interpretations

A. Gram staining technique

The Gram staining reaction is used to help identify pathogens by their Gram reaction (Gram positive or Gram negative) and morphology.

Required reagents

Crystal violet stain→ primary stain

Lugol's iodine → mordant

Acetone–alcohol → decolorizer

Neutral red, 1 g/l (0.1% w/v) → secondary stain

Procedure

1. Prepare a smear from pure fresh colony
2. Fix the smear (alcohol fixation)
3. Cover the fixed smear with crystal violet stain for 30–60 seconds
4. Rapidly wash off the stain with clean water
5. Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds
6. Wash off the iodine with clean water
7. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.
8. Cover the smear with neutral red stain for 2 minutes
9. Wash off the stain with clean water
10. Wipe the back of the slide, and place it in a draining rack for the smear to air-dry
11. Examine the smear microscopically, with the 100x objective to report the bacteria and cells

Interpretation

Gram positive bacteria . . . . Dark purple

Yeast cells . . . . Dark purple

Gram negative bacteria . . . . Pale to dark red

Nuclei of pus cells . . . . Red

Epithelial cells . . . . Pale red

B. Catalase test (tube method)

This test is used to differentiate bacteria that produce the enzyme catalase, such as *Staphylococci*, from non-catalase producing bacteria such as streptococci. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required

Hydrogen peroxide, 3% H_2O_2

Procedure

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod (nota nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

Note: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

3. Look for immediate bubbling as shown in Plate

Interpretation

Active bubbling . . . . Positive catalase test

No bubbles . . . . Negative catalase test

C. Coagulase test

This test is used to identify *S. aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus*: **Free coagulase** which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test. **Bound coagulase** (clumping factor) converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test. *Note:* A tube test must always be performed when the result of a slide test is not clear, or when the slide test is negative

Required

- EDTA anticoagulated human plasma or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Procedure

Slide test method (detects bound coagulase)

1. Place a drop of distilled water on each end of a slide or on two separate slides.
2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

   Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.
3. Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Interpretation

Clumping within 10 secs . . . . *S. aureus*

No clumping within 10 secs. . . . . No bound coagulase

Controls

Positive coagulase control: *Staphylococcus aureus*

Negative coagulase control: *Escherichia coli* or *Staphylococcus epidermidis*

Tube test method (detects free coagulase)

1. Take three small test tubes and label:

   T = Test organism (18–24 h broth culture)*

   Pos = Positive control (18–24 h *S. aureus* broth culture)*

   Neg = Negative control (sterile broth)*

   *Nutrient broth is suitable
2. Pipette 0.2 ml of plasma into each tube.
3. Add 0.8 ml of the test broth culture to tube T.

   Add 0.8 ml of the *S. aureus* culture to the tube labelled ‘Pos’.

Add 0.8 ml of sterile broth to the tube labeled ‘Neg’.

4. After mixing gently, incubate the three tubes at 35–37 ^{0}C. Examine for clotting after 1 hour. If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again.

Note: When looking for clotting, tilt each tube gently.

Interpretation

Clotting of tube contents or fibrin clot in tube . . . . *S. aureus*

No clotting or fibrin clot . . . . Negative test

D. Antimicrobial susceptibility testing (disc diffusion method): *Modified Kirby-Bauer technique*

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

Required

Mueller Hinton agar

Antimicrobial discs

Turbidity standard equivalent to McFarland 0.5

Preparation of turbidity standard

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mix well.

Caution: Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth pipette, and never add the water to the acid.

2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of distilled water.

3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.
4. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula.

When stored in a well-sealed container in the dark at room temperature (20–28 ^{0}C), the standard can be kept for up to 6 months.

Procedure

1. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper.
3. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{0} to ensure even distribution.
4. With the petri dish lid in place, allow 3–5 minutes (no longer than 15 minutes) for the surface of the agar to dry.
5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate. *Note:* The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35 ^{0}C for 16–18 h (24 hours forcefoxitin).
7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the

diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

Interpretation

Antimicrobial disc	Interpretive Criteria (nearest whole mm)		
	Sensitive	**Intermediate**	**Resistant**
Penicillin (10 units)	≥29	–	≤28
Cefoxitin *(30 µg)	≥ 22	–	≤ 21 mm
Erythromycin (15 µg)	≥23	14-22	≤13
Clindamycin (2 µg)	≥21	15–20	≤14
D-test			
Tetracycline (30 µg)	≥19	15–18	≤14
Trimethoprim-Sulphamethoxazole (1.25/23.75 µg)	≥16	11–15	≤10
Rifampin (5 µg)	≥20	17–19	≤16
Vancomycin E-test	≥16 µg/ml	4-8 µg/ml	≤2 µg/ml
Daptomycin E-test	–	–	≤1 µg/ml

* Cefoxitin is used for preliminary screening of MRSA

E. **Protocol for DNA extraction using the DNeasy® Blood & Tissue kit for Gram Positive Bacteria**

Things to do before starting

1. Prepare enzymatic lysis buffer using the following protocol
   - 20 mM Tris-HCl (PH 8.0)
   - 2 mM sodium EDTA
   - 1.2% Triton X-100
   - Immediately before use, add lysozyme (20mg/ml)
2. Preheat a heating block or water bath to 37°C
3. Preheat a heating block or water bath to 56°C

Procedure

1. Harvest bacteria in 1500 µl molecular grade water in a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge for 10 min at 7500 rpm. Discard supernatant
3. Resuspend bacterial pellet in 180 µl enzymatic lysis buffer. Vortex.
4. Incubate for at least 30 min at 37°C. Remove from heating block and vortex afterwards.
5. Add 25 µl proteinase K and 200 µl Buffer AL (without ethanol). Mix by vortexing.
6. Incubate at 56°C for 30 min. Remove from heating block and vortex afterwards.
7. Add 200 µl of 96–100% ethanol to the sample, and mix thoroughly by vortexing.
8. Pipet the mixture (including any precipitate) into the DNeasy Mini spin column.
9. Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.
10. Place the spin column in a new 2 ml collection tube.
11. Add 500 µl Buffer AW1
12. Centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.
13. Place the spin column in a new 2 ml collection tube.
14. Add 500 µl Buffer AW2
15. Centrifuge for 3 min at 14,000 rpm. Discard collection tube.
16. Place the spin column in a clean 1.5 ml microcentrifuge tube.
17. Pipet 100 µl molecular grade water in to spin column.

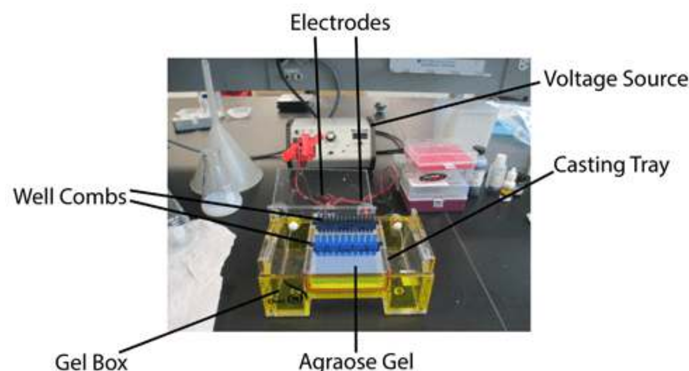
18. Incubate at room temperature for 1 min.
19. Centrifuge for 1 min at 8000 rpm to elute.
20. Store elute at 4 ^{o}C

F. Agarose Gel Electrophoresis

Gel electrophoresis is the standard laboratory procedure for separating DNA by size for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, it is possible to determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

Required materials and reagents

- An electrophoresis chamber
- Power supply
- Gel casting trays
- Sample combs
- Electrophoresis buffer (Tris-acetate-EDTA, TAE)
- Loading buffer
- Ethidium bromide (EtBr)
- Transilluminator



Preparation of 1000ml 1x TAE from 50x TAE stock solution

- Take 20 ml of 50x TAE stock solution in an Erlenmeyer flask and make the volume to 1000 ml by adding 980 ml of distilled water.

Preparation of 2 % Agarose Gel

1 Measure 2 g of agarose
2 Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
3 Microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel).

Note: *It is a good idea to microwave for 30-45 sec, stop and swirl, and then continue towards a boil.*

4. Let agarose solution cool down to about 50°C (about when you can comfortably keep your hand on the flask).
5. Add Ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 μg/mL (usually about 2-3 μl of lab stock solution per 100 mL gel). EtBr binds to the DNA and allows to visualize the DNA under ultraviolet (UV) light.

   Note: *Caution EtBr is a known mutagen. Wear a lab coat, eye protection and gloves when working with this chemical.*
6. Pour the agarose into a gel tray with the well comb in place.

   Note: *Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.*
7. Let the poured gel sit at room temperature for 20-30 minutes, until it has completely solidified.

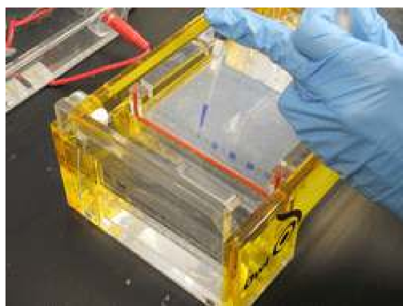
Loading Samples and Running an Agarose Gel:

1. Add loading buffer to each of the PCR product samples.

   Note: *Loading buffer serves: 1) to provides a visible dye that helps with gel loading and allows gauging how far the gel has run; 2) the glycerol in it increases the density of the DNA sample causing it settle to the bottom of the well, instead of diffusing in the buffer.*
2. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
3. Fill gel box with 1xTAE (or TBE) until the gel is covered.

   Note: *Remember to add EtBr to the buffer. EtBr is positively charged and will run the opposite direction from the DNA. So if you run the gel without EtBr in the buffer you will reach a point where the DNA will be in the bottom portion of the gel, but all of the EtBr will be in the top portion and your bands will be differentially intense.*
4. Carefully load a molecular weight ladder into the first lane of the gel.

5. Carefully load your samples into the additional wells of the gel.



6. Run the gel at 100 V for 100 minutes until the dye line is approximately 75-80% of the way down the gel.

   Note: *Black is negative, red is positive. (The DNA is negatively charged and will run towards the positive electrode.)* ***Always Run to Red.***

7. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

8. Place the gel directly on a transilluminator to visualize DNA fragments (bands).

   Note: *When using UV light, protect your skin by wearing safety goggles or a face shield, gloves and a lab coat.*

Analyzing the Gel

Using the DNA ladder in the first lane as a guide, interpret the bands in the sample lanes.

Annex VII: Material Transfer Agreement (MTA)

Annex VI: Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by the Department of Microbiology, Immunology and Parasitology; College of Health Sciences; Addis Ababa University (hereinafter referred to as "Provider") and The Ohio State University and its investigator Wondwossen Gebreyes (hereinafter referred to as "Recipient") in all transfer of research material (samples, derivatives, and specimens) related to the protocol, Phenotypic and molecular characterization of *Staphylococcus aureus* from human and animals in Mekelle, Northern Ethiopia.

Provider: Department of Microbiology, Immunology and Parasitology; College of Health Sciences; Addis Ababa University

Recipient: The Ohio State University, Columbus, Ohio, USA

1. Provider agrees to transfer to recipient designated (Ohio State University, USA) the following research materials /specimen: Bacterial isolates ("Research Material")

The research material will only be used for research purposes as described in the protocol by Recipient's investigator in designated laboratory for the research project described below, under suitable containment conditions. This Research Material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

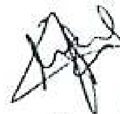
a) Are the research materials of human origin?

Yes ☑ No ☐

b) If yes, are they collected according to the details in the protocol and in adherence to National Research Ethics Review Committee (NRERC) and Addis Ababa University, college of health sciences Ethics Review Committee recommendations and their approval?

Yes ☑ No ☐

2. This research material and its derivatives will be used by Recipient's investigator solely in connection with the following research project ("Research Project") described with specificity as follows: Phenotypic and molecular characterization of *Staphylococcus aureus* from human and animals in Mekelle, Northern Ethiopia.
3. Recipient will acknowledge Provider as the source of the Materials in all publications of results involving the Materials. Neither Party will use the name of, or imply endorsement of, the other or any of such other Party's personnel in connection with any commercial or promotional activities. Recipient will also provide the Research Project results to Provider, including a preview copy of any publication.



OSU Agreement No. MIU · 1021 GEBREYES (MK)

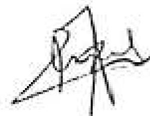
4. This research material represents a significant contribution on the part of Provider and is considered proprietary to Provider. Recipient therefore agrees to retain control over this research Material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of Provider. The research material will be disposed of as agreed upon per protocol at the end of completion of the Research Project.
5. This Agreement may be terminated on the first to occur of the following: 1) completion of the Research Project; or 2) with 30 days prior written notice by either Party. This MTA shall terminate five (5) years from the Effective Date. Recipient will return or dispose of all unused Research Material whenever the Research Project discontinues or is terminated.
6. The Provider does not take any responsibility for loss, damage, wastage or spoilage of the Research Material during or after shipment to the address provided by the Recipient under conditions agreed to in the protocol on shipment of the samples.

This research material is provided as a service to the research community. IT IS BEING SUPPLIED TO RECIPIENT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF ERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the Research Material will not infringe any patent or proprietary right of third parties.

7. Recipient will notify Provider of any Modifications ("Modifications"). "Modifications" means substances created by Recipient that contain or incorporate all or any part of the Research Material. Upon written request, Recipient will provide Provider's providing scientist a sample of Modifications for research purposes within thirty (30) days of such request.
8. Recipient will promptly notify Provider of any inventions or discoveries arising from the Research Project. Ownership will follow inventorship. which will be determined by the relative contributions of the Parties and applicable law.
9. The under-signed provider and recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.
10. Any additional terms (use an attached page if necessary): None.
11. The provider maintains, ownership right of the research material and its unmodified derivatives unless stated otherwise.

The provider will retain a copy (aliquot) of every sample sent abroad as much as possible for local research needs.

The Parties have caused this MTA to be signed as of December 17, 2015 ("Effective Date") by their duly authorized representatives, and warrant the authority of such representative to legally bind such Party. A facsimile or electronically scanned signature will be deemed sufficient evidence of a Party's execution of this MTA.



Material Transfer Agreement

Signature page

For Recipient:

Recipient's Investigator

Wondwossen A. Gebreyes, Ph.D.

Mailing Address for Material:

Department of Veterinary Preventive Medicine

1900 Coffey Rd.

Columbus, OH 43210

Tel: 614-292-9559

Fax: N/A

ERIN BENDER

Director - TCO

Date: DEC 29 2015

Mailing Address for Notices:

1524 North High St.

Columbus, OH 43201

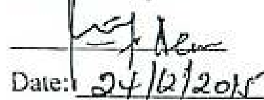
Tel: 614-247-6633

Fax: 614-292-8907

For Provider

Provider's Investigator

Alem Abrha, M.Sc.



Date: 24/12/2015

Mailing Address:

Department of Microbiology, Immunology and Parasitology; School of Medicine, college of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

P.o.Box: 9086

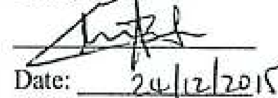
Tel: +215-5528726

Fax: +251-115513099

Duly Authorized

Tamrat Abebe, Ph.D.

Chair, DMIP



Date: 24/12/2015

Mailing Address for Notices:

Department of Microbiology, Immunology and Parasitology; School of Medicine; college of Health Sciences; Addis Ababa University; Addis Ababa; Ethiopia

P.o.Box: 9086

Tel: +215-5528726

Fax: +251-115513099

DECLARATION

I the undersigned declare that this PhD thesis is my own original work and has not been presented for a degree in any other university and all sources of materials used for the thesis have been duly acknowledged.

PhD Candidate: Alem Abrha, B.SC, M.Sc

Signature: _________________

Date of Submission: ______________

Supervisors:

1. Prof. Daniel Asrat (MD, M.Sc, PhD)

   Signature__________________________ Date ___________________

2. Dr. Yimtubezinash Woldeamanuel (MD, M. Sc, PhD)

   Signature______________________________ Date ___________________