ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CLINICAL SAMPLES AT YEKATIT 12 HOSPITAL MEDICAL COLLEGE, ADDIS ABABA, ETHIOPIA

BY

TEBELAY DILNESSA (BSc)

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY SPECIALTY)

ADDIS ABABA, ETHIOPIA

JUNE, 2014

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By:

Tebelay Dilnessa (BSc)

Advisor:

Dr. Adane Bitew (Associate Professor)

A Thesis Submitted to School of Graduate Studies, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty)

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Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

By

Tebelay Dilnessa (BSc)

Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University

Approved by the Examining Board

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Chairman, Dep. Graduate Committee	Signature
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Advisor	Signature
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External Examiner	Signature
_____	_____
Internal Examiner	Signature

I. Acknowledgements

I would like to express my gratitude to Addis Ababa University, College of Health Sciences, School of Allied Health science, Department of Medical Laboratory Sciences for giving me the opportunity to undertake this thesis and funding. My sincere and special thanks also go to my advisor Dr. Adane Bitew for his unreserved guidance, helpful advice and encouragement for the thesis work.

My sincere thanks are to Addis Ababa City Administration Health Bureau Research and Ethics Committee for the approval of my research and writing support letter to Yekatit 12 Hospital Medical College for material support.

I would like to sincerely thank Yekatit 12 Hospital Medical College administration and staffs for allowing me to conduct the research in the hospital. My special thank is to Physicians and Nurses in providing data collection center in Burn unit, ENT, Pediatric, Surgical ward, OPD, etc helping me by informing patients to give specimen and consent by clearly defining the objectives and benefits of participating in the study, and coordinating staffs to stand with me. My heartfelt gratitude and special thank goes to Yekatit 12 Hospital Medical College laboratory case team especially to Ayelign Derbe, Yamirot Merga, Amsalu Tefera and Tigist Tolossa for providing materials, assisting and consulting about the isolation and identification of the microorganisms.

Last but not least, I acknowledge Meseret Assefa (EHNRI), Mulu Hassen (AAU) for their cooperation during beta-lactamase detection of *S. aureus*, Birhan Moges, my classmates and study participants for their consent and time without which the research would not be a reality.

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IV. Abbreviations/ Acronyms

AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
BA	Blood Agar
CA-MRSA	Community Acquired Methicillin Resistant *Staphylococcus aureus*
CDC	Communicable Disease Control and Prevention
CLSI	Clinical Laboratory Standards Institute
CoNS	Coagulase Negative Staphylococci
CSF	Cerebrospinal Fluid
DMLT	Department of Medical Laboratory Technology
DRERC	Department of Research and Ethical Review Committee
EHNRI	Ethiopian Health and Nutrition Research Institute
HA-MRSA	Hospital Acquired Methicillin Resistant *Staphylococcus aureus*
MDR	Multi Drug Resistant
MHA	Muller Hinton Agar
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin Sensitive *Staphylococcus aureus*
PBP	Penicillin-Binding Protein
SOP	Standard Operating Procedure
STGG	Skim-Milk-Tryptone-Glucose-Glycerin
WHO	World Health Organization

V. Abstract

Background: *Staphylococcus aureus* particularly MRSA strains are one of the major causes of community and hospital acquired bacterial infections. They are also becoming increasingly multi-drug resistant, and have recently developed resistance to vancomycin, which has been used successfully to treat MRSA for many years. *In-vitro* determination of drug resistance patterns of *S. aureus* is critical for the selection of effective drugs for the treatment of staphylococci infections.

Objective: The aim of this study was to determine the prevalence of MRSA and MSSA strains isolated from different clinical specimens from patients referred for routine culture and sensitivity testing and also to define the antimicrobial susceptibility pattern of the strains.

Method: A cross sectional study was conducted among 1360 study participants selected conveniently at Yekatit 12 Hospital Medical College from September 2013 to April 2014. Clinical samples from various anatomical sites of the study subjects were collected by employing standard microbiological techniques. Clinical samples were cultured on blood agar and mannitol salt agar and incubated at 35 -37^{0}C aerobically for 18-24 hours. Cultures with typical characteristics of staphylococci were identified to *S. aureus* by using DNAse test. *S. aureus* isolates then were screened for MRSA by using 1μg oxacillin disc. The drug susceptibility patterns of both MRSA and MSSA to twelve antibacterial drugs were determined by disc diffusion procedure. All *S. aureus* isolates examined for beta-lactamase production by employing nitrocefin. Data were coded, entered and analyzed using SPSS version 16 software and logistic regressions were applied to assess any association between dependent and independent variables. P values < 0.05 were taken as statistically significant.

Results: Of the1360 clinical specimens analyzed *S. aureus* was recovered from 194 (14.3%). The prevalence of *S. aurues* was higher in males than females 106 (54.6%) versus 88 (45.4%) and in patients with age group of 25-44 years 64 (33.0%). However, the isolation rate of *S. aureus* was not significantly associated with sex ($p = 0.77$) and age group ($p > 0.05$).

Rate of isolation of *S. aureus* with regards to clinical specimens was the highest in pus 118 (60.8 %) and the lowest in sputum 1 (0.5%). Out of 194 *S. aureus* isolates, 34 (17.5%) were found out to be MRSA and the remaining 160 (82.5%) were MSSA. Relatively a higher MRSA was observed in males than females 19 (55.9%) versus 15 (44.1%) and in the age group 25-44 years 12 (35.3%). No significant association was observed between MRSA and sex (p= 0.87) and age groups (p> 0.05). Ninety eight (50.5%) *S. aureus* were multi-resistant (resistant to three or more antimicrobial agents) and isolates were more resistant to penicillin 187(96.4%) and least resistant for vancomycin 10(5.1%) and cephalothin 6(3.0%). MRSA stains were 100% resistant to penicillin G, erythromycin, trimethoprim-sulfamethoxazole and least resistant to vancomycin 10(29.4%) and cephalothin 6(17.6%). Out of 194 *S. aureus* isolates 153 (79%) were beta-lactames producers. Furthermore, of 34 MRSA isolates 30 (88.2%) and out of 160 MSSA strains 123 (76.8%) produced beta-lactamase.

Conclusion: In this study *Staphylococcus aureus* isolates exhibited very high degree of resistance to different antibiotics. The isolates were also multidrug resistant to several combinations of the tested antibiotics. The emergence of vancomycin resistant *S. aureus* highlights the value of prudent prescribing of antibiotics (including vancomycin) and avoiding their irrational use.

1. Introduction

1.1 Background

Staphylococcal infections still remain an important cause of mortality and morbidity worldwide despite the development of antimicrobial agents. Among the staphylococcus species, *Staphylococcus aureus* is the most virulent species of the genus causing both nosocomal and community acquired infections worldwide [1, 2]. The organism has been found to be the most common bacterial agent recovered from blood stream infections, skin and soft tissue infections, pneumonia and hospital acquired post operative wound infections [3]. If infections caused by *S. aureus* are left untreated, they may lead to bacteremia that could extend to endocarditis, osteomyelitis and septic arthritis [4].

Changes in the drug susceptibility profile of *S. aureus* have been reported worldwide; thereby making treating infections caused by *S. aureus* more difficult [5-7]. Dramatic changes in the susceptibility of *S. aureus* to beta-lactam antibiotics particularly to penicillin and cephalosporin in both hospital and community settings have been reported worldwide [8].

MRSA strains of *S. aureus* were identified immediately up on the introduction of methicillin into clinical practice [9]. The first out breaks of infection caused by MRSA occurred in England in the early 1960s [10]. Since then, strains of MRSA and MSSA staphylococci have spread worldwide [11] and have become established outside the hospital environment, particularly among patients in chronic care facilities and in parenteral drug abusers [12]. The older beta lactams, penicillin and ampicillin are ineffective against more than 80% of the isolated *Staphylococcus aureus* strains and resistance to many of the non beta lactam agents such as tetracycline, gentamycin, chloramphenicol, erythromycin and clindamycin, has gradually increased and reached alarming levels by the 1990s in many parts of the world [13].

Several mechanisms for the development of MRSA have been reported. Among these production of a unique penicillin-binding protein (PBP) that has a low affinity for β-lactam antibiotics and whose effects are determined by several structural genes (mec, mec RI, mec I) [14, 15], production of the usual PBPs, but with modified affinities for the β-lactam drugs, and the production of penicillinase enzyme are the most important ones [16].

MRSA spreads more readily than other strains once introduced into hospitals, and are often difficult to eradicate once established. In some countries MRSA make up to 75% of all *S. aureus* isolates in hospitals [17]. Transmission of MRSA occurs primarily from colonized or infected patients or staff to other patients or staff, or vice versa. Among the resistant pathogens, MRSA is of great concern because of the importance in causing various clinical infections [18].

MRSA has become increasingly prevalent worldwide since it was first reported in British hospital. Prevalence, however, varies markedly in hospitals in the same country and from one country to another. Furthermore, in Ethiopia, little information exists regarding prevalence and drug susceptibility pattern of MRSA and MSSA isolated from various clinical samples. Therefore, studies on the prevalence and drug susceptibility patterns of MRSA and MSSA are of the highest priorities.

1.2. Statement of the Problem

Staphylococci, particularly MRSA strains are one of the leading causes of a variety of human acquired infections (both community and nosocominal infections) for which treatment has proven significantly difficult. The problem is further compounded by the fact that most MRSA strains are also developed resistance to many non beta-lactam antibiotics [19]. To this effect many studies on the prevalence and drug susceptibility pattern of MRSA have been carried worldwide and a significant volume of these worldwide studies depicted that the prevalence of MRSA is increasing. Its rising global incidence is a prime concern for the destabilization of public health. However, the magnitude of studies carried on the prevalence of MRSA incidence in Africa occupies a lower level in contrast to in other part of the world.

In Ethiopia, even though some studies on the prevalence and drug susceptibility pattern of MRSA have been conducted [20-22], the information on the prevalence of MRSA isolated from different clinical samples among Ethiopian patients is inadequate and is not of recent ones. Furthermore, few of these studies dealt with the occurrence of beta-lactamase and multidrug resistance properties of MRSA and MSSA isolated from various clinical samples. Therefore, to comprehensively document the prevalence and antimicrobial susceptibility pattern of both MRSA and MSSA isolated from clinical samples among the Ethiopian patients is of the highest priority.

1.3. Significance of the Study

The results obtained in this study may be used as a baseline data for epidemiological studies of MRSA in the country. Knowledge of the prevalence and antimicrobial susceptibility profiles of MRSA, provides relevant information on the extent of MRSA epidemic, helps to identify infection control mechanisms and selection of appropriate antibiotics for empiric treatment. The insight into the antibiotic susceptibility of clinical isolates profile is very desirable for effective management of the clinical conditions considering the relative differences in the pattern of susceptibility and resistance *Staphylococcus aureus* to antibiotics from one locality to another. Additionally, *Staphylococcus aureus* cultures obtained and maintained in this study will be used for molecular and strain typing study of *S. aureus.*

2. Literature Review

MRSA has become one of the most widespread causes of nosocomial and community acquired infection worldwide. To this effect the prevalence, isolation rate in different clinical samples and drug susceptibility pattern of MRSA have been studied in different part of the world.

A study on methicillin resistance against *S. aureus* in Trinidad & Tobago was conducted by Akpaka et al. [23]. Of 1912 *S. aureus* isolates recovered from different clinical samples 12.8% were found out to be methicillin (oxacillin) resistant. The highest (86%) of the isolates were obtained from wound swabs and the least from urine (0.4%) specimens. As far as the drug susceptibility pattern is considered, 85% of MSSA were sensitive to commonly used antimicrobials in the country. On the other hand, all MRSA isolates were resistant to ceftriaxone, erytheromycin, gentamycin and penicillin but were 100% sensitive to vancomycin, rifampin and chloramphenicol.

Similar study was carried out by Orrett and Land [24] in this country. In this study about 2430 isolates of *S. aureus* strains recovered from various clinical sources, from hospital and community practices were analyzed. The prevalence of MRSA varied with the type of clinical sample. The prevalence of MRSA from surgical/burn wound was the highest (60.1%) followed by urine (15.5%) and pus/abscess (6.6%) respectively. The prevalence of MSSA was also varied with the type of clinical samples. The major sources of MSSA were surgical/burn wounds, pus/abscess and upper respiratory tract specimens with rates of 32.9%, 17.1% and 14.3%, respectively. Furthermore, 109 (4.5%) *S. aureus* strains were isolated from sputum, 201(8.3%) from blood and 95 (4%) from eye infection. Clinical specimens each accounting less than 3% of the total include vagina, ear, CNS. With regard to the antimicrobial susceptibility profile of the isolates the greatest prevalence of resistance of MRSA was seen for erythromycin (86.7%), and clindamycin (75.3%). Resistance rates among MSSA were highest for ampicillin (70%).

Oxacillin resistant and multidrug resistant *Staphylococcus aureus* in Lima, Peru was studied by Seas et al. [19]. *S. aureus* isolates were recovered from blood, sterile body fluids (e.g. cerebrospinal fluid, peritoneal, joint, and pericardial fluids), urine, skin and soft tissue, lungs, abscesses, surgical wound sites, and catheters. Of 103 strains isolated 70 (68%) were MRSA. Studies with regard to the prevalence of MRSA have been carried out in the United States. Prevalence of MRSA in skin and soft tissue infections was conducted by Frazee et al. [25]. Among 137 study subjects, 119 *S. aureus* isolates were recovered of which MRSA was present in 51 % of infection site cultures. Of 119 isolates 89 (75%) were MRSA. All MRSA strains were susceptible to trimethoprim/ sulfamethoxazole, 94% to clindamycin, 86% to tetracycline and 57% to levofloxacin. Similarly, comparison of *S. aureus* from skin and soft tissue infection was carried out by Talan et al. [26]. The results of this study revealed that the prevalence of MRSA was 59%. Similarly Moran et al. [27] conducted MRSA prevalence study in patients with skin and soft-tissue infections. In this study a total of 422 patients with skin and soft-tissue infections were enrolled. *S. aureus* were isolated from skin and soft-tissue infection in 320 (76%) patients of which 249 (78%) of the *S. aureus* isolates were MRSA. This study like other studies has revealed that the isolation rate of MRSA varies with respect to clinical sample. MRSA isolated from abscesses, purulent wounds and cellulitis with purulent exudates accounted 61%, 53% and 47% respectively.

The prevalence of MRSA across the European countries from 1999-2002 was analyzed by Tiemersma et al. [28]. In this study a total of 50,759 *S. aureus* isolates were collected from 495 hospitals in 26 countries. The prevalence of MRSA varied from 1% in Northern Europe to 40% in Southern and Western Europe. The study also has shown that the prevalence of MRSA increased significantly in countries such as Belgium, Germany, Ireland, the Netherlands and United Kingdom while the prevalence of MRSA showed a decrease in Slovenia. In addition this study revealed that MRSA was more frequently isolated from men than women and patients with blood culture positive for MRSA were older than patients with MSSA.

Many studies on the prevalence of MRSA have been conducted in India. A total of 1,426 wound swabs taken from 450 high risk patients were investigated by Vidhani et al. [29] of which bacterial growth was depicted in 407 patients (90.4%). *S. aureus* was isolated from 188 patients (41.8%) out of which 97 patients (51. 6%) was found to be MRSA. A marked difference in antibiotic sensitivity pattern of MRSA and MSSA isolates was reported. According to the results of this study, none of the MRSA isolate was found to be sensitive to penicillin and amoxicillin. However, 6 (5.5%) and 12 (11%) MSSA were sensitive to penicillin and amoxicillin. Eighty-five (77.9%) of MSSA were sensitive to cefotaxime while only 17 (21.5%) of MRSA were sensitive to this antibiotic. Sensitivity to macrolide group of antibiotics like erythromycin and roxithromycin was seen in 77 (70.6%) of MSSA in comparison to 14 (17.7%) of MRSA. Susceptibility test results of this study further showed that amongst the aminoglycosides maximum sensitivity of MSSA was seen with amikacin (74,67.9%) while only 21 (26.6%) MRSA were sensitive to the same antibiotic. Fifty- three (67%) MRSA and 76 (69.7%) of MSSA were found to be sensitive to fluoroquinolone group i.e ofloxacin. All *S. aureus* isolates (MRSA and MSSA) were found to be uniformly sensitive to vancomycin which is the drug of choice for treating infections caused by MRSA.

Another study conducted by Rajendra Goud et al. [30] revealed a prevalence 29.76 % of community associated MRSA. All community associated MRSA were resistant to methicillin and penicillin while resistance to erythromycin and vancomycin was 65% and 1.12 % respectively but, all MRSA isolates were sensitive to Linezolid. A third study conducted by Sharma and Mall [31] found out that out of 200 nasal samples, *S. aureus* was recovered from 97 patients and of these, 23 isolates were MRSA. The drug resistance patterns of MRSA isolated from clinical specimens and carrier screening samples were found to be highly variable. Almost all the MRSA strains (91.3%) screened from nasal samples were resistant to amikacin, 86.95% to kanamycin and cloxacillin, 78.26% to ciprofloxacin, 56.52% to erythromycin, 52.17% to chloramphenicol, and 34.78% to both tetracycline and gentamycin. The production of β-

lactamase enzyme in MRSA was found to be 19 (82.6%). Chandrashekhar et al. [32] isolated 312 *S. aureus* strains of which 177 (56.75%) were found to be methicillin resistant. Results of the drug susceptibility profile of this study showed that all MRSA were resistant to Penicillin, followed by erythromycin (91.5%), amoxycillin (83.6%), norfloxacin (81.4%), cefuroxime (78.5%), amikacin (25.4%). However, no strains were resistant to vancomycin. Similar study carried out by Kaur et al. [3] revealed that 27 out of 70 (38.6%) *S. aureus* isolates were MRSA.

A number of similar studies were carried out in other Asian countries. A study carried out in Tehran by Vahdani et al. [33] exhibited marked variation in the drug susceptibility of MRSA**.** The results of this study showed that all the 90 MRSA isolates were resistant to penicillin (100%), ampicillin (92%) and cefotaxime (93%). Vancomycin and chloramphenicol were the most effective antibiotics and only 7% and 14% of isolates were resistant respectively. Nitrofurantoin, gentamycin, amikacine, ciprofloxacin and other cephalosporins like cefepim and cefazolin were better active than penicillin, ampicillin and cefotaxime. This study showed that 44% of hospital acquired MRSA strains were resistant to cotrimoxazole. Akhter et al. [34] in Karachi isolated MRSA and determined the drug susceptibility of pattern of both MRSA and MSSA. Eighty seven (87) strains of *S. aureus* were recovered from various clinical samples by the authors. Of these, 66 (75.8%) strains were recovered from various swabs and 21(24.13%) from blood. Of the isolates 20 (22.9%) were methicillin resistant. In this group high resistant was found to cloxacillin (100%), co-trimoxazole (95%), erythromycin (70%), gentamicin (55%) and low resistance was observed to ciprofloxacin (30%). In MSSA 0% resistance was seen to ciprofloxacin and chloromycetin and high resistance found to co-trimoxazole (98.5%) and penicillin (73.13%). Both MRSA and MSSA were 100% sensitive to vancomycin. A total of 139 MRSA were isolated by Kaleem et al. [35] in Pakistan**.** Of this most of the MRSA were isolated from pus samples. As far as their drug susceptibility is considered all of the isolated MRSA were found to be susceptible to vancomycin and linezolid. Furthermore, 130 isolates (94%) were susceptible to teicoplanin and minocycline, whereas

93% of isolates were sensitive to chloramphenicol and 91% were sensitive to tetracycline. Only 38 and 22% of the isolates were susceptible to fluoroquinolones and macrolides respectively.

A good number of research work on the prevalence, rate of isolation and drug susceptibility profile of MRSA have been carried out in Africa. A study carried out by Ojulong et al. [36] investigated 188 pus swabs collected from patients with surgical site infections. Out of 54 (28.7%) *S. aureus* isolates, 17 (31.5%) were found out to be MRSA. Resistance rates of MRSA were found out to be 88.2% for trimethoprim-sulfamethoxazole, 88.2% for erythromycin, 58.8% for gentamycin, 70.6% for ciprofloxacin, and 88.2% for chloramphenicol and all MRSA isolates were found to be sensitive to vancomycin and clindamycin. A study carried out in Sudan by Alamin et al. [37] recovered 85 *S. aurues* strains of which 21 (24%) were isolated from nasal cavity, 26 (31%) from skin surface, 22 (26%) from wounds and 16 (19%) from throat. Out of 85 isolates 25 were found out to be MRSA.

Okwu et al. [38] in Nigeria examined 120 samples taken from nose. Of these 22 (18.3%) were found to be positive for *S. aureus* and 13 (10.8%) of the isolates were oxacillin-resistant. Their studies also depicted that 7 (11.7%) MRSA strains were obtained from females while 6 (10%) strains were from males. Also, 12 (19.4%) *S. aureus* and 7 (11.3%) MRSA were isolated from the age group of 9-14 years while 10 (17.3%) isolated of which 6 (10.3%) were MRSA isolated the age groups of 3-8years. Furthermore, the isolates were resistant to ampicillin (100%), cloxacillin (100%), penicillin (100%), tetracycline (82%), chloramphenicol (73%), erythromycin (68%), gentamicin (64%) and oxacillin (55%). Another study conducted by Olowe et al. [39] in the same country, Nigeria depicted that, out of 67 *S. aureus* isolates, 32 (47.8%) were resistant to methicillin. High prevalence of MRSA, 13 (19.4 %) was isolated from wound while urine sample had the least, 1(1.5%). Very high resistance levels (87.5%) were detected against penicillin and tetracycline while gentamicin and vancomycin recorded the least resistance levels of (62.5%) and (6.3%) respectively among the isolates. The starch paper analysis confirmed the presence of beta-lactamase production in all the isolates tested (100%). Similar study

conducted to detect beta-lactamase production in the same country by Efuntoye et al. [40], of the 95 isolates tested 79 (83.2%) were beta-lactamase-producing strains.

In Ethiopia, a retrospective study on the prevalence of MRSA was conducted by Geyid et al. [22]. The results of this study showed that among 249 *S. aureus* isolates 75 (30.5%) were found out to be MRSA while 173 (69.5%) were MSSA. With regards to antibiotic susceptibility pattern of the isolates vancomycin and clindamycin were effective against all *S. aureus* isolates. The presence of beta-lactamase production was determined in the 355 *S. aureus* isolates and 252 (71%) were found to be beta-lactamase procedures. Furthermore, 47 (62%) of the MRSA isolates and 140 (81%) of the MSSA isolates were beta-lactamase positive strains. The sensitivity pattern of all the *S. aureus* isolates against 11 common drugs indicated that the majority (80%) of the MRSA strains were multiple drug resistant while 4 (8%) were not resistant to any of the drugs tested. Forty one (54%) MRSA strains were both beta-lactamase producers and multiple-drug resistant isolates. Another study carried out in Felege Hiwot Referral Hospital, Bahir Dar showed that 55% of *S. aureus* isolates were MRSA [41].

3. Objectives

3.1. General Objective

- To determine the prevalence of MRSA among isolates of *Staphylococcus aureus* recovered from various clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

3.2. Specific Objectives

- To identify and determine the rate of isolation of MRSA and MSSA from different clinical samples referred to culture and sensitivity
- To determine the pattern of antimicrobial susceptibility of MRSA and MSSA isolates
- To determine the production of beta-lactamase in *Staphylococcus aureus*

4. Materials and Methods

4.1. Study Area and Period

The study was conducted at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia from September 2013 to April 2014. It is a tertiary level referral and teaching hospital administered by Addis Ababa City Health Bureau. The hospital consists of an operating room, intensive care unit and 13 wards with a bed capacity of 349. It provides health care services to patients in and around Addis Ababa, the capital city of the country.

4.2 Population

4.2.1 Source Population

The source population was patients referred to Yekatit 12 Hospital Medical College. That is, all inpatients and outpatients who come to the hospital for medical help during the study period were taken as source population.

4.2.2 Study Population

Patients from which clinical samples were taken within the study period

4.3 Study Design

A cross-sectional study was conducted to determine the prevalence of MRSA and MSSA and their drug susceptibility profile.

4.4 Sample Size and Sampling Technique

The sample size was determined using convenient sampling technique which was limited by time from September 2013 to April 2014. Therefore, a total of 1360 study participants were enrolled in the study.

4.5 Measurement

4.5.1 Dependent Variables

- Prevalence of *Staphylococcus aureus*
- Methicillin resistant pattern of *S. aureus* (Susceptible and Resistance)
- Antimicrobial susceptibility pattern of *Staphylococcus aureus*

4.5.2 Independent Variables

- Age
- Sex
- Specimen type

4.5.3. Inclusion and Exclusion Criteria

Inclusion criteria

- All patients seeking routine culture and sensitivity testing and consented to participate in this study was included

Exclusion criteria

- Patients who took antimicrobials within the past two weeks were excluded

4.6 Data Collection and Processing

Specimen collection

Different clinical samples were collected from patients by employing standard microbiological procedures. Nasal swab, pus from wound, ear discharge, blood, throat swab, eye swab, vaginal discharge, urethral discharge, urine, stool, sputum, CSF and body fluids were clinical specimens collected. All specimens were transported to microbiology laboratory of the hospital with minimum delay for culture and sensitivity tests.

Isolation and Identification

Clinical specimens were inoculated on to blood agar base (Oxoid, Basingstoke, Hampshire, England) to which 5% sheep blood was added and mannitol salt agar (Oxoid, Basingstoke, Hampshire, England) by using streaking method. Inoculated plates were incubated at 35-37^{o}c for 18 to 24 hours aerobically. Bacterial colonies showing typical characteristics of *S. aureus* (i.e., beta hemolytic on blood agar and colonies with golden yellow pigmentation on mannitol salt agar) were subjected to subculture on to basic media, gram stain and biochemical tests like catalase and coagulase tests. Catalase positive and gram positive bacteria appearing in grape like cluster was spot inoculated to DNase agar (Oxoid, Basingstoke, Hampshire, England). Inoculated DNAse agar plates were incubated at 37^{0}C over night and flood with 1N HCl (Merk, Darmstadt, Germany). Isolates that hydrolyzed DNA in DNAse agar were considered *S. aureus*.

Detection of MRSA and Determination of Antimicrobial Susceptibility Profile of each isolate

Antimicrobial susceptibility test was carried out by the Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI - formerly NCCLS) guidelines on Muller Hinton agar (Oxoid, Basingstoke, Hampshire, England [42]. Four to five bacterial colonies were inoculated into 5ml of soyabean casein digest broth (Oxoid, Basingstoke, and Hampshire, England) and incubated at35 - 37^{0}C for 24 hours. After an overnight incubation the growth suspension was prepared in 0.5 ml of the same broth medium and the turbidity was adjusted to match that of 0.5 McFarland standards to obtain approximately the organism number of 1x 10^{6} colony forming units (CFU) per ml. Then a sterile swab was dipped into the suspension and the excess of inoculum was removed by pressing it against the sides of the tube. Then the swab was applied to the center of Muller Hinton agar plat and evenly spread on the medium. Antibiotic discs were placed after 15 minute of inoculation to Muller Hinton agar seeded with each isolate and were incubated for 24 hours at 35 - 37^{o}C. The diameter of the zone of inhibition around

the disc was measured using sliding metal caliper. Interpretation criteria were those of Clinical Laboratory Standards Institute (CLSI) guidelines.

The following drugs and concentrations (in brackets) were used to determine the antibiogram of the strains: pencillin [10U], augmentin [30µg], trimethoprim-sulfamethoxazole [1.25/23.75µg], oxacillin [1µg], clindamycin [30µg], gentamicin [30µg], ciprofloxacin [5µg], erythromycin [15µg], chloramphenicol [30µg], cefuroxime [30µg], cephalothin [10µg] and vancomycin [30µg]. All antibiotic disks were obtained from Oxoid Limited Company, United Kingdom and susceptible strain of ATCC 25923 was used as the control strain for identification and susceptibility tests.

Beta-lactamase Production Determination

All *S. aureus* strains were screened for beta-lactamase production by employing the procedures of Efuntoye et al. [40]. Culture of each isolate was streaked onto sticks impregnated with nitocefin a chromogenic cephalosporin (Unipath Limited, Hampshire, England) that produces a rapid color change from yellow to pink/red when the beta-lactam ring is hydrolyzed by beta-lactamase. Therefore, production of beta-lactamase was detected by a change in the color of the stick from yellow to red within 10 minutes.

Culture maintenances

All *S. aureus* isolates were inoculated into STGG (skim-milk-tryptone-glucose-glycerin) medium and were kept at -70^0C.

4.7. Data Processing and Analysis

Data was cleaned, coded; double entered and analyzed using SPSS, version 20. Descriptive statistics, binary and multiple logistical regressions were used to estimate crude and adjusted odds ratio (ORs) with

95% confidence interval (CI) to the different variables. P-value < 0.05 was considered significant. Tables and graphs were used to describe the results.

4.8. Data Quality Assurance

Site assessment was done prior to data collection. All data quality control tools (pre-analytical, analytical and post-analytical stages) of quality assurance that were incorporated in standard operating procedures (SOPs) of the microbiology laboratory were strictly followed. Adequate specimen was collected using appropriate equipment and method. The specimen was kept free of contamination. All materials, equipment and procedures were adequately controlled. Culture media was tested for sterility and performance. The performance of equipments (autoclave, incubators) was monitored by using standard procedures. The data were checked for completeness and representativeness prior to entry. The reliability of the study findings were guaranteed by implementing quality control (QC) measures throughout the whole processes of the laboratory work. S. *aureus* (ATCC 25923) is a methicillin susceptible strain which was used to check that the conditions were favorable for the detection of resistance carried out in this study. The inoculums size during drug susceptibility testing was monitored by using 0.5 McFarland standards [46].

4.9. Ethical Considerations

The study was conducted after it was ethically reviewed and approved by the Department of Research and Ethical Review Committee of Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Ethical clearance was also obtained from Addis Ababa Health Bureau. Then a letter informing the hospital was written from the health bureau and permission was obtained from Yekatit 12 Hospital Medical College. Informed written consents were obtained from participants before data collection. The respondent was given the right to refuse to take part in the study as well as to withdraw at any time during the study period. All the information obtained from the study

subjects were coded to maintain confidentially. When the participants were found to be positive for *S. aureus,* they were informed by the hospital clinician and received proper treatment.

4.10. Dissemination of Results

The result of the study was submitted to Department of Medical Laboratory Sciences, Addis Ababa University. Oral presentation of the thesis was made. Reports was also be submitted to Yekatit 12 Hospital Medical College, Addis Ababa Health Bureau, annual conferences of professional societies and other concerned bodies. Since it is said that scientific work is incomplete until published, the manuscript will be submitted to peer reviewed journals for publication.

5. Results

5.1. Age and Sex Distribution of Study Participants

A total of 1360, study participants were enrolled in the present study of which 654 (48.1%) were males and 706 (51.9%) females with a sex ratio of 0.93:1. The ages of study subjects ranged from one month to 89 years with a mean age of 23.4±0.5 years and median age of 21±0.5 years. The majority of study participants were in age group of 1-14 years 461(33.9%) and 25-44 years 377 (27.7%) (Table 1).

Table 5.1: Age and sex distribution of study participants at Yekatit 12 Hospital Medical College from September 2013 to April 2014

		Number of Samples	
		N	**%**
Sex	Male	654	48.1
	Female	706	51.9
Age group[1]	< 1	105	7.7
	1-14	461	33.9
	15-24	210	15.4
	25-44	377	27.7
	45-64	133	9.8
	65+	74	5.4
	Total	1360	100.0

[1]-WHO age classification for health [47]

5.2. Prevalence of *S. aureus* among Study Participants

Of a total of 1360 clinical samples *S. aureus* was isolated from 194 (14.3%). Males had a higher isolation rate of *S. aureus* than females 106 (54.6%) vs 88 (45.4%). Rate of isolation of *S. aureus* was the highest in 25-44 years 64 (33.0%) (Table 2). As shown in table 3, the isolation rate of S. *aureus* was not significantly associated with sex [AOR, 95% CI: 1.05(0.72, 1.53), p = 0.77] and age group [COR, 95% CI: 1.47(0.55, 3.92), p > 0.05].

Table 5.2: Distribution of *S. aureus* in study participants with gender and age group at Yekatit 12 Hospital Medical College from September 2013 to April 2014

		Number of sampled	**Presence of *S. aureus***	
		n (%)	**Yes (n/%)**	**NO (n/%)**
Sex	Male	654 (48.1)	106 (54.6)	548 (47.0)
	Female	706 (51.9)	88 (45.4)	618 (53.0)
Age group	< 1	105 (7.7)	9 (4.6)	96 (8.2)
	1-14	461 (33.9)	48 (24.8)	413 (35.2)
	15-24	210 (15.4)	46 (23.7)	164 (14.0)
	25-44	377 (27.7)	64 (33)	313 (26.8)
	45-64	133 (9.8)	18 (9.3)	115 (9.8)
	65+	74 (5.4)	9 (4.6)	65 (5.5)
	Total	**1360 (100.0)**	**194 (100.0)**	**1166 (100.0)**

Table 5.3: Association of *Staphylococcus aureus* in study participants with regard to gender and age group at Yekatit 12 Hospital Medical College from September 2013 to April 2014

		Presence of *S. aureus*		**P**	**COR**	**95% CI**	**P**	**AOR**	**95% CI**
		Yes (n/%)	**No (n/%)**						
Sex	Male	106(54.6)	548 (47.)	0.05	0.736	(0.54, 0.99)			(0.72,
							0.771	1.057	1.53)
	Female	88(45.4)	618(53.0)						
Age	< 1	9 (4.6)	96 (8.2)	0.434	1.477	(0.55, 3.92)			
group	1-14	48 (24.8)	413(35.2)	0.651	1.191	(0.55, 2.54)			
	15-24	46 (23.7)	164(14.0)	0.072	0.494	(0.22, 1.06)			
	25-44	64 (33)	313(26.8)	0.306	0.677	(0.32, 1.42)			
	45-64	18 (9.3)	115(9.8)	0.779	0.885	(0.37, 2.08)			
	65+	9 (4.6)	65 (5.5)	-	-	-			

COR: crude odds ratio, AOR: adjusted odds ratio

Isolation Rate of *Staphylococcus aureus* from Clinical Samples

In our study the major sources of *S. aureus* were pus/abscess, ear discharge, blood, nasal swab and throat swab which together, accounted for 175(90.2%) of all isolates (Table 4). The lowest isolates were found from sputum 1(0.5%). The rest of the isolates were from urine, vaginal discharge, eye swab, body fluid, stool and sputum, accounting < 10.0% of the total. No *S. aureus* was isolated from CSF and urethral discharge. As can be depicted from table 5, recovery rate of *S. aureus* was significantly associated with pus [AOR, 95% CI: 67.07(9.1, 493.8), p= 0.000], nasal swab [AOR, 95% CI: 27(3.19, 228.05), p= 0.002], throat swab [AOR, 95% CI: 11.12(1.3, 94.37), p= 0.027] and ear discharge [AOR, 95% CI: 21.2(2.8, 162.3), p= 0.003].

Table 5.4: Isolation rate of *Staphylococcus aureus* from different clinical specimens at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Type of Specimen	**Total Sample**		***S. aureus* Isolated**	
	N	**%**	**N**	**%**
Pus/abscess	213	15.7	118	60.8
Nasal swab	27	2.0	9	4.6
Throat swab	41	3.0	7	3.6
Vaginal discharge	121	8.9	3	1.5
Urethral discharge	15	1.1	0	-
Urine	217	16.0	6	3.1
Eye swab	16	1.2	2	1.0
Blood	223	16.4	17	8.8
Body fluid	75	5.5	4	2.0
Stool	171	12.6	3	1.5
Ear discharge	85	6.3	24	12.4
CSF	101	7.4	0	-
Sputum	55	4.0	1	0.5
Total	**1360**	**100.0**	**194**	**100.0**

Table 5.5: Isolation Rate of *Staphylococcus aureus* (Multivariate analysis) from different clinical specimens at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Specimen Type	Presence of *S. aureus*		Total	P-Value	AOR	95% CI
	Yes (N/%)	No (N/ %)	N/%			
Pus/abscess	118(60.8)	95(8.1)	213(15.7)	0.000**	67.074	(9.11, 493.82)
Nasal swab	9(4.6)	18(1.5)	27(2.0)	0.002**	27.000	(3.19, 228.05)
Throat swab	7(3.6)	34(3.0)	41(3.0)	0.027*	11.118	(1.31, 94.37)
Vaginal discharge	3(1.5)	118 (10.1)	121(8.9)	0.786	1.373	(0.14, 13.50)
Urine	6(3.1)	211 (18.1)	217(16.0)	0.694	1.536	(0.18, 13.02)
Eye swab	2(1.0)	14(1.2)	16(1.2)	0.105	7.714	(0.65, 91.32)
Blood	17(8.8)	206(17.7)	223(16.4)	0.151	4.456	(0.58, 34.23)
Body fluid	4(2.0)	71(6.0)	75(5.5)	0.326	3.042	(0.33, 28.00)
Stool	3(1.5)	168(14.4)	171(12.6)	0.975	0.964	(0.09, 9.46)
Ear discharge	24(12.4)	61(5.2)	85(6.3)	0.003**	21.246	(2.78, 162.35)
Sputum	1(0.5)	54 (4.6)	55 (4.0)	-	-	-

** Significant at P value < 0.01, * Significant at P value < 0.05, AOR: adjusted odds ratio

5.3. Prevalence of MRSA

Out of 194 *S. aurues* recovered, 34 (17.5%) were found out to be MRSA and the remaining 160 (82.5%) were MSSA (Figure 1). Relatively a higher MRSA were isolated in males than females 19(55.9%) versus 15(44.1%) and the highest MRSA was detected in the age group 25-44 years 12(35.3%) followed by age group 15-24 years 8(23.5%), age group1-14 years 7(20.6%), age group 45-64 years 5(14.7%) and the least in age group above 65 years 2(5.9%). As can be seen in table 6, isolation rate of MRSA in relation

to gender was not significantly associated [COR, 95% CI: 0.94(0.44, 1.98), p= 0.87] as well as any of age groups [COR, 95% CI: 1.23(0.22, 6.7), p > 0.805] (Table 6).

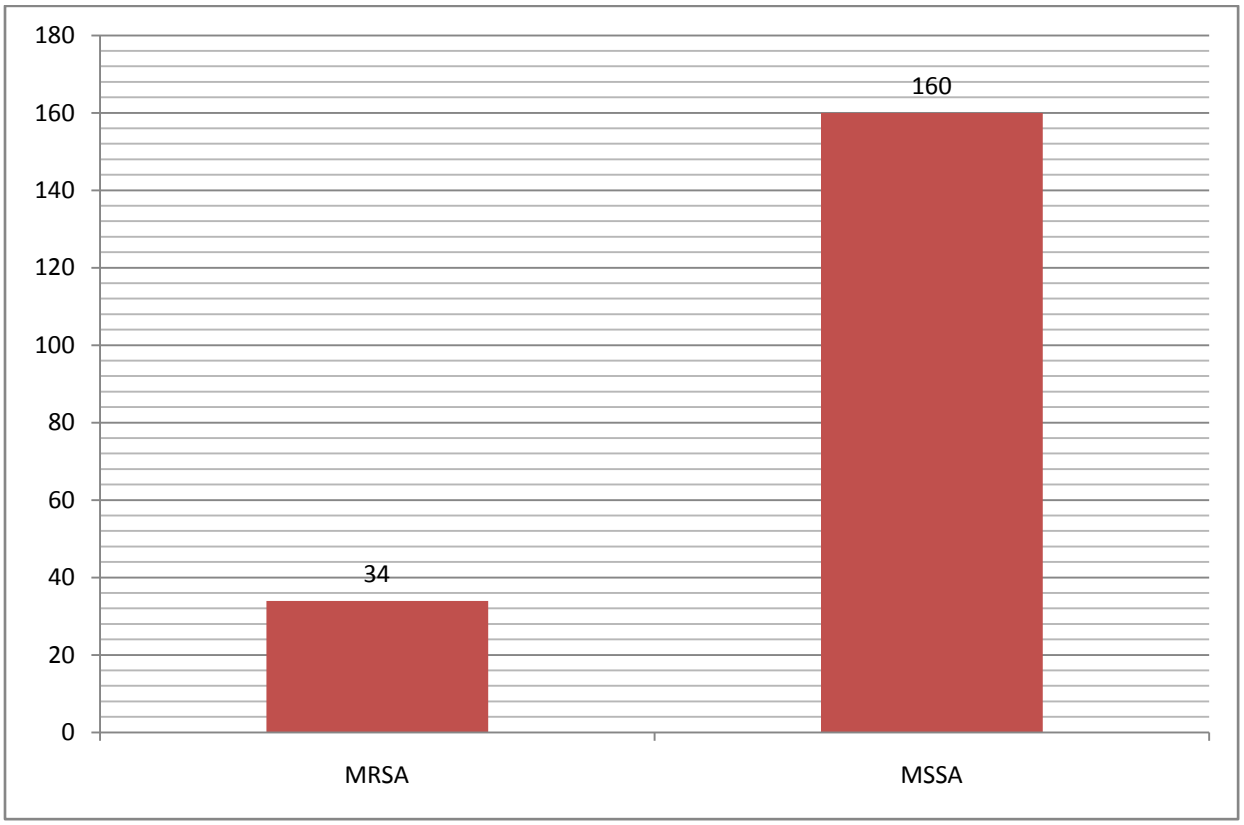

Figure 5.1: Methicillin Susceptibility Pattern of *Staphylococcus aureus* at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Table 5.6: Association of Methicillin Resistant Pattern of *S. aureus* in study participants with gender and age group at Yekatit 12 Hospital Medical College from September 2013 to April 2014

		MRSA		MSSA		P-value	COR	95% CI
		N	%	N	%			
	Male	19	55.9	87	54.4	0.873	0.941	(0.44, 1.98)
Sex	Female	15	44.1	73	45.6			
	< 1	0	-	9	5.6	0.999	-	-
Age	1-14	7	20.6	41	25.6	0.567	1.673	(0.287, 9.76)
group	15-24	8	23.5	38	23.8	0.732	1.357	(0.23, 7.78)
	25-44	12	35.3	52	32.5	0.805	1.238	(0.22, 6.72)
	45-64	5	14.7	13	8.1	0.757	0.743	(0.11, 4.86)
	65+	2	5.9	7	4.4			

The major source of MRSA was pus 24(70.6%), nasal swab 3(8.8%), ear discharge 3(8.8%), throat swab 2(5.9%) and blood 2(5.9%). No MRSA was observed in urine, stool, body fluid, eye swab, sputum and vaginal discharge. Similarly, pus 94(58.7%) had high rate of MSSA followed by ear discharge 21(13.1%) and blood 15(9.4%). As can be seen in table 7, no statistical association existed between isolation rates of MSRA with any of clinical samples ($p > 0.05$).

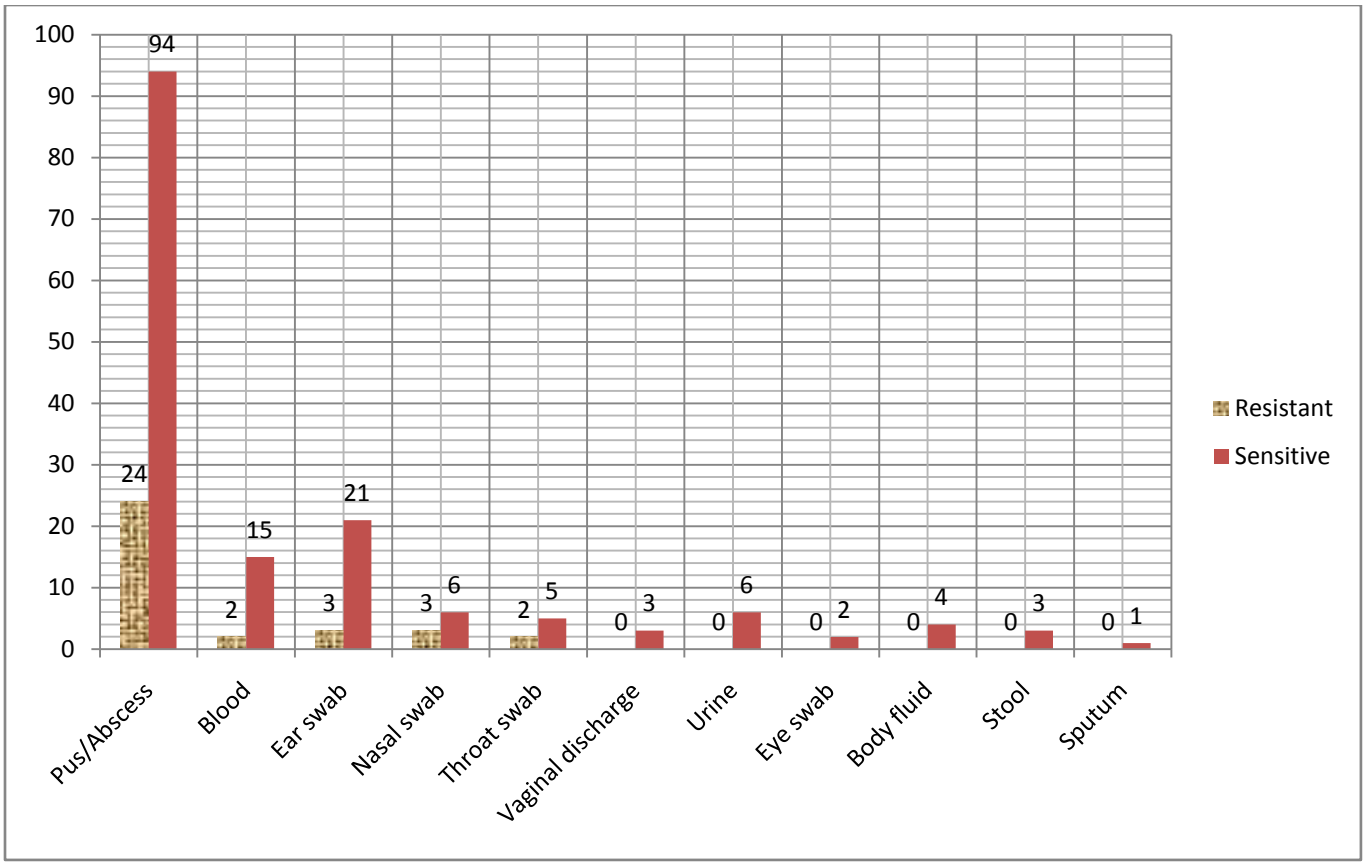

Figure 5.2: Methicillin Resistant Pattern of *Staphylococcus aureus* in Different Clinical Specimens at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Table 5.7: Association of Methicillin Resistant *Staphylococcus aureus* to different clinical specimens at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Type of Specimen	MRSA		MSSA		P-value	COR	95% CI
	N	%	N	%			
Pus/abscess	24	70.6	94	58.7	0.366	0.511	(0.11, 2.19)
Nasal swab	3	8.8	6	3.7	0.605	0.638	(0.11, 3.49)
Throat swab	2	5.9	5	3.1	0.999	0.000	0.000
Vaginal discharge	0	-	3	1.9	0.999	1.000	0.000
Urine	0	-	6	3.7	0.409	1.915	(0.41, 8.95)
Eye swab	0	-	2	1.2	0.999	0.000	0.000
Blood	2	5.9	15	9.4	0.999	1.000	0.000
Body fluid	0	-	4	2.5	0.999	1.000	0.000
Stool	0	-	3	1.9	1.000	0.000	0.000
Ear discharge	3	8.8	21	13.1	0.378	1.787	(0.49, 6.49)
Sputum	0	-	1	0.6			

COR: crude odds ratio

5.4. Antimicrobial Susceptibility Pattern of *Staphylococcus aureus*

Antimicrobial profile of *S. aurues* isolated in this study is presented in table 8 were highly resistant to penicillin 187(96.4%), trimethoprim-sulphamethoxazole 103(53.1%), erythromycin 103(53.1%) and ciprofloxacin 61(31.4%). On contrary, lower resistant was manifested by gentamicin 26(13.4%), clindamycin 23(11.9%), augmentin 18(9.3%), cefuroxime 16(8.2%), vancomycin 10(5.1%) and cephalothin 6(3.0%).

Table 5.8: Antimicrobial Susceptibility Pattern of *S. aureus* strains to different antimicrobial Agents at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Antibiotics [ ]	**Resistant**		**Sensitive**	
	N	**%**	**N**	**%**
Oxacillin [1μg]	34	17.5	160	82.5
Augmentin [30μg]	18	9.3	176	90.7
Penicillin G [10U]	187	96.4	7	3.6
Vancomycin [30μg]	10	5.1	184	94.9
Trimethoprim-Sulfamethoxazole [1.25/23.75μg]	103	53.1	91	46.9
Chloramphenicol [30μg]	36	18.6	158	81.4
Gentamycin [10μg]	26	13.4	168	86.6
Cefuroxime [30μg]	16	8.2	178	91.8
Clindamycin [30μg]	23	11.9	171	88.1
Ciprofloxacin [5μg]	61	31.4	133	68.6
Cephalothin [10μg]	6	3 .0	188	97.0
Erythromycin [15μg]	103	53.1	91	46.9

None of the isolates were 100% susceptible to any one of the drugs and 98 (50.5%) of the isolates were multi-resistant (resistant to three or more antimicrobial agents). MRSA isolates were 100% resistant for penicillin, erythromycin, trimethoprim-sulfamethoxazole and least resistant for vancomycin 10 (29.4%) and cephalotin 6 (17.6%). On the other hand, 153(95.6%) of MSSA were resistant to penicillin (Table 9).

Table 5.9: Association of Methicillin Resistant and Sensitive *S. aureus* to different antimicrobial agents at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Antibiotics [ ]	**MRSA (n=34)**		**MSSA (n=160)**		**P-value**	**AOR**	**95% CI**
	n	**%**	**N**	**%**			
Augmentin [30µg]	15	44.1	3	1.8	0.015*	9.809	(1.56, 61.69)
Penicillin G [10U]	34	100	153	95.6	0.99	0.000	0.000
Vancomycin [30µg]	10	29.4	0	0	0.99	0.000	0.000
Trimethoprim-Sulfamethoxazole [1.25/23.75µg]	34	100	69	43.1	0.135	16.247	(0.41, 630.28)
Chloramphenicol [30µg]	16	47.0	20	12.5	0.923	1.130	(0.09, 13.63)
Gentamycin [10µg]	13	38.2	13	8.1	0.931	0.896	(0.07, 10.98)
Cefuroxime [30µg]	15	44.1	1	0.6	0.000**	216.173	(10.80, 432.2)
Clindamycin [30µg]	18	53.0	5	3.1	0.007**	13.223	(1.99, 87.62)
Ciprofloxacin [5µg]	28	82.5	33	20.6	0.598	0.615	(0.10, 3.73)
Erythromycin [15µg]	34	100	69	43.1	0.136	9.044	(0.49, 164.05)
Cephalothin [10µg]	6	17.6	0	0	-	-	-

** Significant at P value < 0.01, * Significant at P value < 0.05, AOR: adjusted odds ratio

The highest multi-drug resistant were occurred among penicillin G, erythromycin, trimethoprim-sulfamethoxazole 18(18.4%) followed by penicillin G, erythromycin, trimethoprim-sulphamethoxazole and ciprofloxacin 13(13.26%).Thirty (30.64%) of multi-drug resistant was observed by triple drugs. Pencillin resistant was seen in all multi-drug resistant strains (Table 10).

Table 5.10: Antibiograms (Multi drug resistant) of the total *Staphylococcus aureus* isolates at Yekatit 12 Hospital Medical College from September 2013 to April 2014

Antibiotics	**Resistant Strains**	
	Number	**%**
P, E, SXT	18	18.40
P, G, E	5	5.10
P, C, SXT	4	4.08
P, C, CIP	3	3.06
P, CIP, E, SXT	13	13.26
P, G, C, E	3	3.06
P,G, C, SXT	2	2.04
P, CIP, E, SXT	1	1.02
P, AMC, E, SXT	1	1.02
P, OX, G, SXT	1	1.02
P, OX, E, SXT	2	2.04
P, C, CIP, E, SXT	2	2.04
P, G, CIP, AMC, SXT	1	1.02
P, G, CIP, E, SXT	1	1.02
P, G, C, E, SXT	1	1.02
P, CIP, E, SXT, DA	2	2.04
P, OX, C, E, SXT	1	1.02
P, G, C, CIP, CXM, SXT	1	1.02
P, G, C, CIP, E, SXT	3	3.06
P, G, C, AMC, E, SXT	1	1.02
P, OX, CIP, E, SXT, DA	3	3.06
P, OX, C, E, CXM, SXT	2	2.04
P, G, C, CIP, E, SXT, DA	2	2.04
P, OX, G, C, CIP, E, SXT	2	2.04
P, OX, C, CIP, E, CXM, SXT	5	5.10
P, OX, C, E, CXM, SXT, DA	1	1.02
P, OX, CIP, AMC, KF, E, CXM	2	2.04

P, OX, CIP, AMC, KF, SXT, DA	2	2.04
P, OX, G, C, CIP, E, CXM, SXT	2	2.04
P, OX, VAN, CIP,AMC, E, SXT, DA	5	5.10
P, OX, VAN, CIP, AMC, E, CXM, SXT, DA	1	1.02
P, OX, C, CIP, AMC, E, CXM, SXT, DA	1	1.02
P, OX, VAN, CIP, AMC, KF, E, SXT, DA	1	1.02
P, OX, VAN, G, C, CIP, AMC, E, SXT, DA	1	1.02
P, OX, VAN, G, C, CIP, AMC, E, CXM, SXT, DA	2	2.04
Total	**98**	**100.00**

P = Penicillin G, CIP = Ciprofloxacin; AMC = Amoxicillin/clavulanic acid; C = Chloramphenicol; OX = Oxacillin; KF = Cephalothin; E = Erythromycin; G = Gentamycin; VAN =Vancomycin; SXT = Trimethoprim-sulphamethoxazole; DA =Clindamycin; CXM= Cefuroxime

5.5 Beta-lactamase Production in *Staphylococcus aureus*

Of 194 *S. aureus* isolates, 153 (79.0%) were beta-lactames producers. Furthermore of 34 MRSA isolates 30 (88.2%) and out of 160 MSSA strains 123 (76.8%) produced beta-lactamase.

6. Discussion

The present study showed that males had a higher isolation rate of *Staphylococcus aureus* than females. Rate of isolation of *S. aureus* was also the highest in 25-44 years of age group. Prevalence of MRSA in the present study, however, did not vary significantly by gender ($p = 0.87$) and age group ($p > 0.05$) and this is in agreement with earlier reports by Geyid et al.[22] indicating that gender and age are not risk factor for the acquisition or colonization of MRSA.

In this study, the prevalence of MRSA was found to be 17.5%. The prevalence of MRSA recorded in our study was less than that had been reported in Addis Ababa [20, 22, 48] and outside Addis Ababa [41]. Many similar studies have reported a marked variation in the prevalence rates of MRSA in hospitals of the same country. For example, a prevalence rates of 0.7% in 1992– 1993; 4.8% in 1995–1996; 9.8% in 1997–1998 and 12.8 % in 2006 had been reported in Trinidad & Tobago [49-52]. Different studies have also depicted variations in the prevalence rates of MRSA in different countries. Over 50% prevalence rate of MRSA was reported in Portugal and Italy; 25% in England, Greece and France; 2% in the Netherlands and Switzerland [52]. Prevalence of MRSA ranged from 23.6% in Australia to over 61% in Taiwan and Singapore, and more than 70% in Japan and Hong Kong [53]. Differences in the length of study period, number of study sites, sample size, sample type and the laboratory procedures employed may be factors that could contribute to variations in the prevalence rate of MRSA [23]. The rate of MRSA obtained in this study however, was nearly the same as MRSA prevalence rate recorded in a pan-European data that was obtained from studies conducted among 43 laboratories from 10 European countries [54].

Although no statistical association exited between isolation rates of MSRA and MSSA with any of clinical samples ($p > 0.05$), the present study depicted that prevalence of MRSA and MSSA isolated from pus was the highest as compared to other clinical samples. This finding was in agreement with the result obtained in Ethiopia [22, 41] and many similar studies [23, 24, 35] conducted in other parts of the world.

A highest isolation rate of *S. aureus* in general and MRSA in particular in pus in our study could partly be due to the fact that most of the wound samples came from surgical wards and burn unit of the hospital. MRSA on surgical wards is becoming increasingly common especially in critically ill patients who have spent prolonged periods on the intensive care unit [24, 54]. Even though this study was not designed to identify risk factors for MRSA acquisition, risk factors that have previously been associated with acquisition of MRSA in hospitals such as broad-spectrum antimicrobial therapy, admission to an intensive care unit, older age and proximity to other patients with MRSA [55-57] could play a major role in our study site.

Drug susceptibility test on all the 194 *S. aureus* isolates against twelve commonly used antibiotics indicated that 187 (96.4 %) were resistant to penicillin and this finding was in agreement with the findings of Abera et al. [41]. The lowest drug resistant was observed for vancomycin 10 (5.1%) and cephalothin 6 (3%). Furthermore, 98 (50.5%) of the isolates were multi-drug resistant (resistant to three or more antibiotics).

All MRSA isolates encountered in this study were completely resistant (100%) to antibiotics such as penicillin, erythromycin and trimethoprim-sulfamethoxazole. Similar results were noted for penicillin among MRSA strains in Brazil, Chile, Mexico [58], India [32] Trinidad & Tobago [23]. Unlike most studies in Ethiopia [20, 22] and elsewhere in the world [23, 36] vancomycin resistant was very high. Ten (5.1%) out of 194 *S. aureus* isolates were resistant to vancomycin and of 34 MRSA, 10 (29.4%) were vancomycin resistant. MRSA that are also resistant to vancomycin was ranged from 0% in Ethiopia, Karachi and Uganda [22, 34, 36] to (8%) in Iran, Malaysia and Nigeria [30, 37, 39] have been reported. A similar result was obtained for vancomycin in previous reports in Trinidad [50, 51, 59], and MRSA isolates from Argentina, Brazil, Chile, Mexico and Uruguay [58]. Diekema et al [53] have reported that most MRSA strains are resistant to most other antibiotics, thereby necessitating the use of glycopeptides antibiotics, such as vancomycin. Treatment failure has been incriminated as a cause of decreased

susceptibility of staphylococci to vancomycin [60, 61].Wise use and continuous surveillance susceptibility testing of MRSA against vancomycin have been reported as a remedy to control reduced susceptibility of staphylococci to vancomycin [62-65].

Several mechanisms for the development of bacterial drug resistance have been reported. Among these mechanisms, production of a unique penicillin-binding protein (PBP) that has a low affinity for β-lactam antibiotics [14, 15] and production of penicillinase are the most important ones [16]. In the present study all *S. aureus* isolates were tested for beta-lactamase production. It has been shown that out of 194 isolates 153 (79.0%) were beta-lactames producers. Beta-lactamase producing strains of *S. aureus* in the present study were much higher than that has been reported by previous study conducted in Ethiopia [22] but more or less the same as reported in India [31] and Nigeria [40]. Our study further depicted that out of 34 MRSA strains 30(88.2%) produced beta-lactamase and out of 160 MSSA strains 123 (76.8%) were found out to be beta-lactamase producers.

7. Limitations of the Study

Main limitations are as follows:

- More sensitive and specific molecular techniques could not be used to identify the species and strain typing of *S. aureus*
- The infection is due to community or hospital acquired strains could not be identified
- The study was hospital based which may decrease/increase the detection rate of *S. aureus*. It is better including the community as well.
- Clinical data could not be taken which might be important to characterize the association of *S. aureus* to it.

8. Conclusions and Recommendations

Conclusion

The prevalence of *S. aureus* and MRSA varies appreciably based on type of clinical samples. Pus/abscess is the main source of *S. aureus* and MRSA than other samples in hospital settings. The prevalence of MRSA stains obtained in this study was low when compared with the prevalence rates obtained in previous studies conducted in Ethiopia. However, the prevalence rate is considerable high when compared to other similar studies conducted elsewhere. Many MRSA strains were multidrug-resistant and a good number of the isolates were also resistant to vancomycin, the drug of choice for treating multidrug resistant MRSA infections.

Recommendations

Based on the results, the following points are recommended:

- Physicians better prescribe drugs after the sensitivity pattern of the microbe is known
- Additionally large scale l study is needed to determine CA-MRSA and HA-MRSA
- Further phenotypic and genotypic studies are needed to establish and clarify the genetic mechanism behind susceptibilities to antibiotics.
- Drugs such as gentamicin, augmentin, clindamycin, cefuroxime, and cephalothin are recommended for empirical treatment for *S. aureus* infection.
- It is necessary to establish an antimicrobial susceptibility surveillance system to improve current infection control programs in hospitals to prevent the spread of MRSA.
- Reducing this burden by good infection control practices such as strict hand washing, by identifying MRSA carriers and treating them and prudent use of antimicrobial agents is recommended.

9. References

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10. Annexes

Annex I: English Versions of Participant Information Sheet

My name is Tebelay Dilnessa. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled prevalence and drug susceptibility pattern of methicillin resistant *S. aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College.

You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

Introduction

The topic of this study is "**Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia**". Since methicillin resistant *Staphylococcus aureus* is one of the major health problems in our country, the result of the study can be helpful in planning and intervention to solve the prevailing problem. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the hospital with no problem. If you decide to participate, you have to sign on the assent/ permission template form and you may obtain a copy of this information sheet.

Expected from participants

As a participant of this study, you are expected to give nasal swab/ pus/ ear discharge/blood/sputum, etc. Being asked to give sample does not neccessarly mean that you have the disease.When you are found to be positive for the micro-organism, you will be informed by the health worker and receive proper treatment. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required

You will spend 10-15 minutes until the specimen is collected and permission form is signed.

Risks of participant

Specimen collection will have no effect and you will not get any risk as the sample will be collected by well trained professionals. But you may fill minor temporary pain during sample collection.

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access the information. The information will be encoded in a computer and saved with password protection.

Benefits of participation

By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of methicillin resistant *Staphylococcus aureus* infection. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are:

Investigator: Tebelay Dilnessa (BSc), DMLS; AAU, Mobile +251912198715

Email- tebelay@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU Mobile +251911039162

Email-bitewadane@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170

Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign,please understand purpose of the study, procedure,risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have question. I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Agree to participate? Yes---------- No----------

Annex II: Amharic Versions of Participant Information Sheet

እኔ ተበላይ ድልነሳ እባላለሁ፡፡ በአዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ፣ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ስራየን በመስራት ላይ እገኛለሁ፡፡ በመሆኑም እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ በጥናት ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ፡፡

መግቢያ

የጥናቱ ርዕስ **“ስታፊሎኮከስ አሪስ የተባለዉ ደቂቅ ህዋስ ሜቲሲሊን ለተባለዉ መድሀኒት ያለዉ ተላምዶ እና ስርጭት እንዲሁም ይሄው ባክቴሪያ በአብዛኛዉ ለምንጠቀምባቸዉ መድሀኒቶች ያለዉ ተላምዶ በየካቲት 12 ሆስፒታል ህክምና ትምህርት ቤት ለታደሙ ህመምተኞች፤ አዲስ አበባ፤ ኢትዮጵያ”** በሚል ርዕስ እያጠናሁ እገኛለሁ፡፡ ይህ ጥናትም በተሳታፊ ሙሉ ፈቃደኝነት ላይ ተመስርቶ ስታፊሎኮከስ አሪስ የተባለዉ ባክቴሪያ ለመድሀኒቶች ያለዉን ተላምዶ ስርጭት ለማወቅ እና አማራጭ መንገዶችን ለመጠቆም ያስችላል፡፡

ከጥናቱ ተሳታፊ የሚጠበቁ

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙና (ደም፤ የአፍንጫ ጥራጊ፤ አክታ፤ የቁስል ንክዝ፤ ዉሀ ሽንት ወዘተ…) እንዲወሰድ እና ለጥናቱ እንዲዉል መስማማት ይጠበቅቦታል፡፡ የጤና ባለሙያ ከእርሰዎ ናሙናዉን ይሰበስባል፡፡ ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል፡፡ ይሁን እንጂ ይህ ዓይነቱ መረጃ የእርሶን ማንነት የማገልጹ ማስረጃዎን ማለትም ስም፣ አድራሻና የመሳሰሉት መረጃዎች አይጨምርም፡፡ ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ ናሙና ሰጡ ማለት በሽታው ይገኝብዎታል ማለት አይደለም፡፡ በእርሰዎ ናሙና ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡፡

ተሳታፊዉ የሚያጠፋዉ ጊዜ

የተዘጋጀዉን የስምምነት ቅጽ ለመፈረምና ናሙና ለመስጠት 10-15 ደቂቃ ያስፈልጋል፡፡

በጥናቱ በመሳተፍ የሚስከትላቸዉ ችግሮች

ናሙና በሚሰበሰብበት ወቅት ምነም አይነት ችግር አያስከትልቦትም፡፡ ሆኖም ናሙናዉ በሚወሰድበት ጊዜ ትንሽ የህመም ስሜት ሊኖር ይችላል፡፡

የመረጃዉ ሚስጥራዊነት

ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦረቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡ ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ፡፡ ከዚህም በላይ ስለ እርስዎ ያለዉን ማንኛዉንም መረጃ የይለፍ-ቃል ባለዉ የኮፒዉተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል፡፡

በጥናቱ በመሳተፍ የሚያስከትላቸዉ ጥቅሞች

ይህ ጥናት የማስተርስ ዲግር መመረቂያ እንደመሆኑ መጠን በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማጥቅም የለም። ለወደፊት በተመሳሳይ ሁኔታ ዉስጥ ላሉ በሽተኞች በመረጃ ላይ የተመረተ ህክምና ለመስጠት ያግዛል። ከፈለጉ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ እንዲሁም ስለ አስፈላጊዉ ህክምና ከሀኪምዎ ጋር ይነጋገራሉ።

የጥናቱ ተሳታፊዎች መብት

ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተና ተሳትፎዎን መተውና በማንኛውም ሰዓት ጥናቱን ማቆም ይችላሉ።በጥናቱ ዉስጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማቋረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠቅና ገለፃ የማግነት መብት አለዎት። የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል።

ግንኙነትና ጥያቄ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥዉ ድንገተኛ ችግር ወይም ጥያቄ ካሎት በሚከተለዉ አድራሻ ይጠቀሙ።

ተመራማሪ፣ ተበላይ ድልነሳ (ቢ.ኤስ.ሲ) ሞባይል +251912198715, ኢ-ሜይል፤ tebelay@gmail.com

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ

አማካሪ፣ አዳነ ቢተዉ (ፒ.ኤች. ዲ) ሞባይል +251911039162, ኢ-ሜይል፤ bitewadane@gmail.com

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ፤ የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ።

ስልክ-+251112755170

ከዚህ በታች የሚገኘው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን፣ መስማትዎን እና መገንዘብዎን የሚያሳይ ነው። ከመፈረምዎ በፊት እባክዎትን የጥናቱን ዓላማ፣ የተሳትፎ ጉዳትና ጥቅሙ፣ የመተው፣ የማቋረጥ፣ መብትና ነፃነት እንዳለዎት ይረዱ። ተስማምተዋል? የጥናቱን መግለጫ አንብቢያለሁ/ ሰምቻለሁ እናም ተረድቻለሁ። መመሪያው ምን እንደ ሆነና በእኔ ምን ሊከሰት እንደሚችል ተረድቻለሁ። በጥናቱ ላይ ለመሳተፍ፤

እስማማለሁ ---------- አልስማማም ----------

Annex III: English Versions of Consent form

This page contains an agreement signature to participate in the study entitled with "**Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia"**. So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study "Prevalence and antimirobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College"
2. I know that the information/ specimen that I will give used for this study only.
3. I understand that, all the information given for the study and the results are confidential.
4. I understand that I will not get any money for my participation.
5. I understand that I have a right to stop from participation any time in the study.
6. I understand all the information which is explained by specimen collector/Nurse.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Please direct any questions or problems you may encounter during this study to:

Tebelay Dilnessa

Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Mobile +251912198715

Email- tebelay@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170

Annex IV: Amharic Versions of Consent form

የተሳታፊ ስምምነት ቅጽ

ይህ ገጽ **"Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia"** ማለትም ስታፊሎኮከስ አሪስ የተባለዉ ደቂቅ ህዋስ ሜቲሲሊን ለተባለዉ መድሀኒት ያለዉ ተላምዶ እና ስርጭት እንዲሁም ይሄው ባክቴሪያ በአብዛኛዉ ለምንጠቀምባቸዉ መድሀኒቶቺ ያለዉ ተላምዶ በሚል ርዕስ የተሳታፊ ስምምነት ቅጽ ነዉ፡፡ በመሆኑም እባክዎን በዚህ በታች የተዘረዘሩትን ነጥቦች ይረዱና፤ ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ፡፡

1. እኔ "ስታፊሎኮከስ አሪስ የተባለዉ ደቂቅ ህዋስ ሜቲሲሊን ለተባለዉ መድሀኒት ያለዉ ተላምዶ እና ስርጭት እንዲሁም ይሄው ባክቴሪያ በአብዛኛዉ ለምንጠቀምባቸዉ መድሀኒቶቺ ያለዉ ተላምዶ" የሚለዉ ጥናት አላማ በደንብ ተገንዝቤአለሁ፡፡
2. ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ፡፡
3. ሁሉም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ፡፡
4. በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ፡፡
5. በጥናቱ ያለመሳተፍ እንዲሁም በማንኛዉም ጊዜ የማቃረጥ መብት እንዳለኝ አዉቄአለሁ፡፡
6. ሁሉም መረጃዎች በአስተባባሪዉ/ዎች ተገልጾልኝ በደንብ ተረድቻለሁ፡፡

የተሳታፊ ፊርማ፡ ----------

የተሳታፊ አድራሻ፡----------

ቀን፡----------

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ተበላይ ድልነሳ ሞባይል +251912198715

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይል፤ tebelay@gmail.com

ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ፡፡ ስልክ+251112755170

Annex V: Media Preparation, Procedure for Specimen Collection and Processing

Blood Agar

Blood agar is a bacterial growth medium which contains 5% sheep's blood. It is considered to be differential but not selective, because it is an enriched medium that provides a rich nutrient environment for many types of bacteria, while a selective medium supports the growth of certain types of bacteria but inhibits other types. It is used to distinguish pathogenic bacteria based on the effect of bacterial enzymes known as hemolysins which lyse red blood cells.

Preparation of blood agar

1. Measure 1000ml of distilled water into a liter conical flask.
2. Weigh 40g of Blood Agar Base.
3. Add and suspend the measured BA into the 1000ml of distilled water.
4. Heat with frequent agitation and boil for one minute to completely dissolve the powder.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 45-50°C and aseptically add 50ml of sterile defibrinated blood.

   NB: Blood is taken from sheep from jagular vein and collected to a vessel which contains beads that defibrinate the bood
7. Arrange the petri-dishes onto the clean safety hood and then gently pour (18-20ml) the warm blood agar onto the plates.
8. Cover the petri-dishes and allow the blood agar to coagulate before storage in a refrigerator.
9. Label on the bottom of the blood agar plates the name of media, preparation date, expiration date and store at 2-8^{o}c

Mannitol Salt Agar (MSA)

Mannitol salt agar is a differential and selective media. It is selective because its high salt concentration (7.5 %) inhibits the growth of most bacteria. However, Staphylococcus is able to tolerate this high salinity. MSA is differential because it contains the sugar mannitol and phenol red, a pH indicator. When mannitol is fermented, acid products are produced and the pH drops. Phenol red is yellow in color below pH 6.8. Thus, mannitol fermenters such as *S. aureus* will have a yellow halo around them. Mannitol non-fermenters such as *Staphylococcus epidermidis* will leave the MSA media unaltered (pink).

Preparation of mannitol salt agar

1. Measure 1000ml of distilled water and add into a conical flask.
2. Weigh 111g of Mannitol salt agar powder.
3. Add and suspend the measured MSA into the 1000ml of distilled water.
4. Heat with frequent agitation and boil for one minute to completely dissolve the powder.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 45-50°C for dispense
7. Arrange the petri-dishes onto the clean safety hood and then gently pour (18-20ml) onto the plates.
8. Cover the petri-dishes and allow the media to coagulate before storage in a refrigerator.
9. Label on the bottom of the plates name of media, preparation date and expiration date and store at 2-8^{o}c

Mueller Hinton Agar (MHA)

Mueller Hinton Broth is a general-purpose medium that may be used in the cultivation of a wide variety of fastidious and non-fastidious microorganisms. Additionally, in recent times this media has been used in standardized antimicrobial disk susceptibility testing. The Kirby-Bauer antimicrobial disk diffusion procedure is used with Mueller Hinton Agar plates. It is based on the use of an antimicrobial impregnated filter paper disk. The impregnated disk is placed on an agar surface, resulting in diffusion of the antimicrobial into the surrounding medium. Effectiveness of the antimicrobial can be shown by measuring the zone of inhibition for a pure culture of an organism. Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganisms.

Preparation of Mueller Hinton Agar

1. Measure 1000ml of distilled water into a conical flask.
2. Weigh 21g of mueller hinton agar powder.
3. Add and suspend the measured powder into the 1000ml of distilled water. Mix thoroughly.
4. Heat with frequent agitation and boil until completely dissolve the powder.
5. Sterilize by autoclave at 121°C for 15 minutes under 15 Ibs pressure and cool to 45-50°C overnight
6. Arrange the petri-dishes onto the clean safety hood and then gently pour the media onto the plates.
7. Test the sterility by incubating some media at 37^{o}C for 24 hrs
8. Label with name of media, preparation date, expire date, and store at 2-8 ^{o}C for maximum two months

A. Collection and processing of specimen from burn/ pus wound infection

1. The specimen will be collected by an experienced nurse and special care will be taken to avoid contaminating the specimen with commensal organisms from the skin
2. With sterile cotton tipped applicator stick moistened with normal saline collect sample from the infected site
3. Label the sample with the patient code number and send to lab as soon as possible
4. Inoculate in to mannitol salt agar and blood agar aseptically
5. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
6. Examine and report the culture; look for colony characteristics and perform biochemical tests
7. Determine drug susceptibility pattern of the isolated organism

B. Collection and processing of Nasal swab

1. With a sterile cotton swab moistened with sterile normal saline gently swab the inside of noses
2. Label the sample with the patient code number and send to lab as soon as possible
3. Inoculate the specimen in to mannitol salt agar and blood agar aseptically
4. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
5. Examine and report the culture; look for colony characteristics and perform biochemical test
6. Determine drug susceptibility pattern of the isolated organism

C. Collection and processing of Blood sample

Blood cultures can be obtained either by using a needle and syringe or by the closed system using a vacuum bottle and the double-needle collection tube.

1. Clean the sample collection area (about 50 mm in diameter) using 70% ethanol
2. About 10 ml of blood from an adult or about 2-5 ml from a young child is collected using standard venous blood collection
3. Incubate 2-4 days at 35^{o} to 37^{o} and observe if there is turbidity
4. Inoculate the specimen in to mannitol salt agar and blood agar aseptically
5. Examine and report the culture; look for colony characteristics and perform biochemical test
6. Determine drug susceptibility pattern of the isolated organism

D. Collection of Urethral Discharge

1. Insert a sterile swab (cotton wool-tipped on a plastic stick) about 30 mm into the urethral canal
2. Gently rotate it against the wall of the urethra
3. Place the swab in a dry tube and deliver it to the laboratory
4. Inoculate the specimen in to mannitol salt agar and blood agar aseptically

5. Examine and report the culture; look for colony characteristics and perform biochemical test
6. Determine drug susceptibility pattern of the isolated organism

E. Collection of Throat swab

1. The patient is instructed to tilt his/her head back and breathe deeply
2. The tongue is gently depressed with a tongue blade to visualize the tonsillar fossa and posterior pharynx
3. The swab is extended between the tonsillar pillars and behind the vulva, care should b taken not to touch the lateral walls of the buccal cavity
4. The posterior pharynx should be firmly rubbed with the swab
5. After collection, the swab should be placed immediately into sterile tube
6. Inoculate the specimen in to mannitol salt agar and blood agar aseptically
7. Examine and report the culture; look for colony characteristics and perform biochemical test
8. Determine drug susceptibility pattern of the isolated organism

F. Collection of Eye swab

1. Pull down the lower eyelid so that the lower conjunctival fornix is exposed
2. Swab the fornix without touching the rim of the eyelid with the sterile cotton swab
3. Place the swab immediately in a bacterial transport medium or in a sterile test tube with 0.5 ml of buffered saline (pH 7)
4. Inoculate the specimen in to mannitol salt agar and blood agar aseptically
5. Examine and report the culture; look for colony characteristics and perform biochemical test
6. Determine drug susceptibility pattern of the isolated organism

G. Collection of Ear discharge

1. Collect a specimen of the discharge on a thin, sterile cotton wool or Dacron swab
2. Place the swab in a container with the transport medium, breaking off the swab stick to allow the stopper to be replaced tightly
3. Label the specimen and send it to the laboratory
4. Inoculate the specimen in to mannitol salt agar and blood agar aseptically
5. Examine and report the culture; look for colony characteristics and perform biochemical test
6. Determine drug susceptibility pattern of the isolated organism

H. Biochemical testing procedures

Identification of gram positive bacteria: Gram-positive cocci will be identified based on their gram reaction, catalase, coagulase and DNAse positive test results.

Procedure for Catalase test

This test is used to differentiate *staphylococci* (+ve) from *streptococci (*-ve).

Principle: 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 in to 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.

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. Using a sterile wooden stick take the organism and immerse into the hydrogen peroxide solution
3. Look for immediate bubbling
4. Interpretation

Active bubbling **----------**positive test

No release of bubbles **----------** negative test

Procedure for Coagulase test

This test is used to differentiate *Staphylococcus aureus* from other staphylococcus species.

Principle: In the presence of the enzyme coagulase, the addition of commercial rabbit plasma produces a clumping reaction.

1. Place a drop of physiological saline on two separate slides
2. Emulsify the test organism in each of the drop to make thick suspension
3. Add one drop of plasma to one of the suspensions and mix gently
4. Look for clumping of the organism within 10 seconds
5. Interpretation

Clumping within 10 seconds **----------***S. aureus*

No clumping within 10 seconds **----------**other Staphylococcus *species*

Procedure for DNAse test

This test is used to help in the identification of *S. aureus* which produces DNAase enzymes.

1. Divide a DNA-ase plate into the required number of strips by marking underside of the plate
2. Using a sterile loop or swab, spot-inoculate the test and control organisms
3. Incubate the plate at 35–37^{o}C overnight
4. Cover the surface of the plate with 1 mol/l hydrochloric acid solution
5. Look for clearing around the colonies within 5 minutes of adding the acid
6. Interpretation

Clearing around the colonies . . . . . DNA-ase positive strain

No clearing around the colonies . . . . . DNA-ase negative strain

I. Antimicrobial Sensitivity Testing Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth
2. Match the turbidity of suspension with turbidity standard (0.5 MacFarland standard)
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of test tube to remove the excess fluid).
4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab
5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
6. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
7. Measure the radius of the inhibition zone by caliber
8. Interpret the reaction of the test organism to each antibiotics used as sensitive or resistance as per the standard

J. Beta-lactamase production test

1. Each nitrocephin impregnated rods is touched with colony on the agar plate
2. A positive reaction is shown by the development of a pink/red color visible within 5 minutes
3. A negative reaction is indicated by no color change within 15 minutes and the absence of beta-lactamase

K. Criteria of specimen rejection

- Inappropriate specimen transport device
- Mislabeled specimen
- Unlabeled specimen
- Specimen received after prolonged delay (usually more than two hour)
- Specimen received insufficiently

Annex VI: Data Collection Forms

Socio-demographic and Methicillin Susceptibility Pattern (R &S)

ID number	Type of Specimen	Age	Sex	Coagulase	DNAse	*S. aureus* status	Methicillin Susceptibility

Drug Susceptibility Pattern of *S. aureus* to various drugs (R&S)

ID number	Specimen type	OX	CXM	DA	CN	KF	AMC	CIP	SXT	VA	C	P	E

Interpretive criteria drug resistant tests based on CLSI guidelines (mm)

Antibiotics	**Susceptible**	**Intermediate**	**Resistant**	**Remark**
Oxacillin [1µg]	≥13	11 - 12	≤ 10	
Augmentin [30µg]	≥ 20	-	≤ 19	
Penicillin G [10U]	≥29	-	≤28	
Vancomycin [30µg]	≥ 11	-	≤ 12	
Trimethoprim-Sulfamethoxazole [1.25/23.75µg]	≥16	11 - 15	≤10	
Chloramphenicol [30µg]	≥18	13 - 17	≤12	
Gentamycin [10µg]	≥15	13 - 14	≤12	
Cefuroxime [30µg]	≥18	15 - 17	≤14	
Ciprofloxacin [5µg]	≥21	16 - 20	≤15	
Cephalothin [10µg]	≥18	15 - 17	≤14	
Erythromycin [15µg]	≥23	14 - 22	≤13	
Clindamycin [30µg]	≥21	15 - 20	≤14	

Annex VII: Declaration

Title of Project: Prevalence and Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia.

I the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in this or any other University and all sources of materials used for this thesis have been acknowledged.

Name: **Tebelay Dilnessa (BSc)**

Address: Department of Medical Laboratory Sciences, Addis Ababa University

Signature: _____

Date of Submission: _____

This thesis has been submitted with my approval as **University advisor**.

Name: **Adane Bitew (MSc, PhD)**

Address: Department of Medical Laboratory Sciences, Addis Ababa University

Signature: _____

Date of Submission: _____