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The Prevalence and Anti-microbial susceptibility patterns of MRSA among patients suspected for Hospital acquired infections in admitted patients and associated risk factors in TikurAnbessa specialized hospital,Addis Ababa.

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This is to certify that the thesis prepared by TewodrosTamire, entitled: The Prevalence and Anti-microbial susceptibility patterns of MRSA among patients suspected for hospital acquired infections in admitted patients and associated risk factors in TikurAnbessa specialized hospital, Addis Ababa *Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostics and public health Microbiology specialty) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.*

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II. Acknowledgement

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III. Abstract

Background: *Staphylococcus aureus* is a gram-positive bacterium that can cause community acquired and hospital acquired infections. Development of antimicrobial resistance has limited treatment options against infections due to this pathogen.

Objectives: To assess the prevalence of methicillin resistance Staphylococcus aureus among patients suspected for HAI and associated risk factors in admitted patients.

Methods: A cross-sectional study was conducted from January 2018 to January 2019 to determine the prevalence of Meticillin Resistance *Staphylococcus aureus* and associated risk factors Among Admitted Patients at TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia. Samples were cultured for *S. aureus isolations* according to (CLSI 2018). Isolates were tested for susceptibility to panels of 14 antimicrobial agents using disc diffusion assay. Susceptibility to methicillin was phenotypically determined based on sensitivity of isolates to cefoxitin or oxacillin.

Result: Out of 413 patients, a total of 160(38.7%) Coagulase positive *S. aureus and 59(14.3%) Coagulase negative* isolates were recovered from patients involved in the study. Out of 160 isolates, 57 were MRSA. All 57 isolates of MRSA are 100% resistance to Cefoxitin, penicillin, augumentin,Carbapenems and Cephalosporins. And 28.1%(16) resistant to Clindamycin,80.7%%(46) to Ciprofloxacin,91.2%(52) to Gentamycin, 61.4%(35) to Erythromycin, and 65%(37) Trimethoprim-Sulfamethoxazole respectively. Majority of the coagulase positive isolates exhibited (n=160, 38.7%) multi drug resistance. The overall burden of MRSA among the admitted patients were 57/413(13.8%). The prevalence of MRSA among the isolates in males and females' patients are 35(61.4%) and 22(38.6%) respectively. Duration of hospital admission length was not statistically significant in the acquisitions of MRSA in Chi-square test (where P value<0.05) and the mean staying length in the hospital was approximately 6 days.

Conclusion: *S. aureus* isolates from patients in TikurAnbessa specialized hospital exhibited resistance to antibiotics most commonly used for the treatment of staphylococcal infections. Hence, there is an urgent need to strengthen infection prevention and control in the wards.

Key terms: MRSA, ICUs, Surgical site infections, nosocomial infection and Antimicrobial susceptibility patterns, Addis Ababa, Ethiopia.

IV. List of Abbreviations

AST: Antimicrobial susceptibility test

ATCC: American type culture collection

PVL: Panton Valentine leucocidin

BHS: Beta hemolytic *Staphylococcus*

Cops: Coagulase positive *Staphylococcus*

Cons: Coagulase negative *Staphylococcus*

EARSS: European Antimicrobial Resistance Surveillance System

HAI: Hospital Acquired Infections

ICU: Intensive Care Unit

MRSA: Methicillin Resistant *Staphylococcus aureus*

MSSA: Methicillin Susceptible *Staphylococcus aureus*

MHA: Muller Hinton Agar

MIC: Minimum inhibitory concentration

NLF: Non lactose fermenters

PBP: Penicillin binding protein

PCR: Polymerase chain reaction

SSI: Surgical Site Infection

TSST-1: Toxic Shock Syndrome Toxin-1

NHL: non-Hodgkin's lymphoma

QC: Quality control

VRSA: Vancomycin resistant *Staphylococcus aureus*

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1. Introduction

1.1 Background

Staphylococcus aureus, non-motile, non-spore forming, coagulase-positive, gram-positive bacterium, with grape like cluster is among the most successful human pathogens. Both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) can cause mild to fatal diseases, spread locally and globally, colonize numerous human body parts, and persist in various environments outside of hosts (1, 2).

It is also a commensal that colonizes the anterior nares of asymptomatic carriers, who may unknowingly transmit the pathogen within the community or within healthcare facilities.Since the introduction of β-lactam antibiotics, Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to one of a leading cause of healthcare-associated infections (1, 2).

The route of transmission is generally accepted as patient-to-patient from contaminated healthcare workers' hands. While hands are transmission vectors, the major reservoir for contamination is from colonized or infected individuals. These patients can transfer the pathogen directly to healthcare workers following skin-to-skin contact as well as shed the pathogens on their desquamated skin cells onto the environment, resulting in environmental contamination. Clean hands then become contaminated by touching the contaminated surfaces (1,3).

The presence of colonized or infected patients is known to affect acquisition risks of other patients. Colonization pressure is a measure of both exposure magnitude as well as exposure time. A systematic review of studies using colonization pressure showed that there have been various definitions of colonization pressure calculated over various lengths of time (one day, three days, one week, and one month) (1).

The measure used to quantify the proportion of patients who are MRSA–positive who share the same general ward or intensive care unit with others in a given time is called "colonization pressure". This measure was first described in 1994 and has since been recognized as a risk factor for nosocomial infections (1).

Data regarding the prevalence and distribution of methicillin-sensitive S. aureus (MSSA) and MRSA in Africa are scarce, and control measures within healthcare settings are limited due to

constraints with respect to resources and diagnostic facilities. Studies on S. aureus carriage by hospital patients in Madagascar report a MRSA prevalence of between 4 and 13%. Madagascan hospitals set no guidelines for screening patients or HCW for MRSA. In most African countries, HA-MRSA constitutes 20-50% of *S. aureus* infections. Different MRSA strains have emerged in hospitals and in the community in both Europe and the USA (1, 2, 4).

Factors such as travel and close contact with animals may contribute to the dissemination of the bacterium in Africa: one MRSA clone in particular (ST-88-MRSA), which is found worldwide, is also widespread in West, Central, and East Africa. A multicenter study conducted across five different African countries identified one major clone, which is also predominant in Madagascar (ST-88 IV, spa type t186). A high prevalence of the staphylococcal virulence factor, Panton-Valentine leucocidin (PVL), was found in MSSA strains isolated from hospital patients from mainland Africa and the Western Indian Ocean region (1).

Evidence suggests that PVL is associated with staphylococcal skin and soft tissue infections. No data are available on the presence of PVL in MSSA and MRSA isolates obtained from HCW in Madagascar. Another staphylococcal virulence factor, toxic shock syndrome toxin-1 (TSST-1), causes severe illness and multi system clinical manifestations. Resistance to β-lactam antibiotics (as in MRSA) is due to the acquisition of the mecA gene. In recent years, a similar gene termed mecC has been described, which is often not detected by routine laboratory tests because standard molecular methods usually focus only on detecting mecA (1, 2,3).

1.2 Characteristics of S. *aureus* infections

S. aureus is a gram positive, cluster-forming coccus, none motile, and non-spore forming anaerobe bacterium. It is also commonly found in extra nasal sites such as the skin, oral cavity, throat, gastrointestinal tract, umbilicus, groin, and per-rectum. It is related to a variety of bacterial strains that can cause clinical symptoms ranging from a single skin lesion to toxic shock syndrome and death (3, 36).

Staph infections have different pathological manifestations in the human body. Two of which are pus-forming infections and toxins. S. aureus frequently generates localized infections at the site of entry because of the active defense mechanisms response of the host immune system. Hair follicles, breaks in the skin's protective layers resulting from the needle stick injuries and

wounds, and the respiratory tract are considered potential sites of entry for human staph infections. The host's immune system responds to infections with inflammation, swelling, supportive discharge, fever, headache and tissue death (1, 36).

1.3 Origin of methicillin resistance

The first report of methicillin resistance was identified in 1961. The specific gene responsible for methicillin resistance was not identified until over 20 years later (1). The structural gene for methicillin resistance, *mec*A, encodes a novel penicillin-binding protein (PBP)-2a (or PBP2`), which has reduced affinity for β-lactam antibiotics. This gene is carried on a mobile genetic element, *Staphylococcal* Chromosomal Cassette (SCC*mec*) (2). The original donor of *mec*A to staphylococci is unknown, as the element has not yet been identified outside this genus. The origin of the cassette SCC*mec* could be from staphylococci other that *S. aureus* (4). It has been suggested that *Staphylococcus sciuri* harbored the ancestor of PBP2a, because the PBP found in *S. sciuri* showed 87.8% amino-acid sequence identity with PBP2a (5). As of 2009, there are eight SCC*mec* types and numerous subtypes described by the International Working Group on the Classification of *Staphylococcal* Cassette Chromosome elements (IWG-SCC) (6).

1.4 Acquisition of MRSA infections

MRSA lives harmlessly on the skin usually in the nose, armpits, groin or buttocks. This is known as colorizations or carrying MRSA. You can acquire MRSA on your skin by:

- By touching someone who has it.
- Sharing things like towels, sheets and clothes with someone who has MRSA on the skin.
- Touching surfaces or objects that have MRSA on them (1,36)

People staying in the hospital are most at risk of having MRSA because:

- They often have a way for the bacteria to get into their body, such as a wound, burn, feeding tube, drip into a vein, or urinary catheter.
- They may have other serious health problems that mean their body is less able to fight off the bacteria.
- They are in close contact with a large number of people, so the bacteria can spread more easily (1, 36).

1.5 Potential Sources and Sites of Infection for S. aureus

The pathogenesis of staph diseases produces broad clinical symptoms. S. aureus can colonize on the human skin without damaging the protective layers of the epidermis. Skin irritation and invasive procedures such as peripheral venous access catheter (intravenous line) insertion, surgically created passageways (arteriovenous fistula) for dialysis, and surgical incisions can alter the protective barriers of layers of skin. Breaks in the skin facilitate the migration of this human pathogen into open wounds, which can then lead to localized or severe infections (1, 2& 36).

Primary indications of S. aureus infection include shallow skin lesions such as infections of the eyelid (sties), formation of pus-filled bumps under the skin (boils), inflammatory conditions affecting hair follicles (folliculitis), localized suppurative skin infection of the hair follicles and subcutaneous tissues (furuncles), highly contagious red sores on the skin (impetigo), and the collection of pus in any part of the body. Complications of secondary infections include invasive forms of bacterial diseases such as lung abscesses, pneumonia, urinary tract infections (UTIs), osteomyelitis, endocarditis, arthritis, meningitis, toxic shock syndrome, food poisoning, septicemia, and death (1, 36).

1.6 Pathogenesis of S. *aureus*

S. aureus is a pathogenic bacterium with virulence factors that can invade immunocompetent and immunocompromised human hosts. This microbe attacks the human immune system by adapting to and resisting innate and adaptive immune responses (1, 36).

Those who experience such bacterial invasion find that their immune systems are compromised and they have various symptoms of infection. S. *aureus* produces different kinds of leukotoxins, which are substances that protect pathogens from human leukocytes (neutrophils). Examples include panton-valentine leukocidin (PVL), gamma-hemolysins (HIgAB and HIgCB), Leukocidin AB, and Leukocidin ED. Leukotoxins target mainly neutrophils, which are the first responders to microbial infections. They also destroy different immune cells by releasing toxins, whereas HIgAB, HIgCB and LukED destroy red blood cells (2, 36).

S. *aureus* first attacks the innate immune cells of the host. After the initial S. *aureus* infection, the adaptive immune system tries to work against the S. *aureus* bacterial invasion. Meanwhile,

lukED destroys CCRS-positive memory T lymphocytes. This process is termed as S. aureus infection (36).

Biological adaptation and mutations have made this human pathogen a very successful infectious agent. The major characteristics of the microbe are resistance to antimicrobial agents such as methicillin, erythromycin, levofloxacin, mupirocin, and tetracycline, and its reduced susceptibility to vancomycin and daptomycin (1, 2, and 36).

Colonization of S. aureus depends on environmental stimuli such as cigarette smoke. Because cigarette smoke, including second hand smoker, suppresses the immune system and facilitates the formation of staph biofilm, oxidative stress, and expression of pathogenic virulence. S. *aureus bacteria* stick to one other inside the nasopharynx, and they compromise fibronectin. Smoking enhances the binding of S. aureus to fibronectin and adherence to human cells (1, 36).

1.7 Management of septic wounds with antibiotics

It has been noted that inappropriate use of antibiotics can lead to development of resistance to antibiotics (7&8). Inappropriate use includes; no indication, incorrect choice, incorrect application of drugs and divergence from institutional guidelines. Antibiotic prophylaxis has been shown to significantly reduce rate of wound infection (11, 12). A ground was laid for antibiotic prophylaxis as early as 1960s (13,14). However, a study done in the United Kingdom showed there was no benefit in using flucloxacillin prophylaxis in patients with open fracture (15).

A systematic review done in New Jersey, found that short course of first generation prophylaxis administered as soon as possible after the injury provided adequate prevention against wound infections (16). A national advisory for prophylaxis recommends use of cefazolin, cefuroxime or vancomycin for knee, hip, cardiothoracic or vascular surgery prophylaxis while for the colon, aminoglycosides, macrolides or metronidazole should be considered (17). Even though antibiotic use in clean wounds is not clearly indicated, infection rates of 40% post-surgery have been reported (9).

Selection of antibiotics should be based on the infecting organism, tissue penetration ability, low toxicity and absence of allergies. In a study carried out in Ireland, the antibiotics that are mostly used are combinations of penicillin’s and beta lactamase inhibitors and macrolides (18). The rate

of using second generation cephalosporin's use was 6% while third generation cephalosporin's were 13%. In another study, cephalosporin's use for antibiotic prophylaxis was at 67% (39). A systematic review found that for MRSA eradication, linezolid performed better than vancomycin however amoxicillin clavulanic offered better prophylaxis against MRSA infections (19, 20). *Klebsiella pneumonia* responds well to polymixin combinations and amino glycosides (21).

A study carried out in Clayton, Australia; found that flucloxacillin continuous infusion offered good activity against wound infections with MSSA (22). Antimicrobial treatment of non-healing polymicrobial and/or clinically infected wounds should be targeted to cover most of the potentially synergistic aerobic or facultative and anaerobic microorganisms and not simply target specific common pathogens e.g. *Staphylococcus aureus* and *Pseudomonas aeruginosa* (23).

The International working group on the diabetic foot recommends intravenous or oral use of empirical broad spectrum antibiotics in deep foot infections. The regimens that can be used include; ampicillin/salbactum, ticacillin/clavulanate, amoxicillin/ clavulanate clindamycin and a quinolone, asecond or third generation cephalosporins with a quinolone or metronidazole with aquinolone" (24).

A study carried out at Cardiff, Wales University found thatantibiotic prescribing for wound infection was based on expert opinion and not scientific facts (7). The antibiotics those were commonly used included; flucloxacillin,,amoxi-clavulinate, cefaclor, cefalexin, erythromycin, trimethoprim, metronidazole and ciprofloxacin. Flucloxacillin, and metronidazole were mostly prescribed.

A study carried out at the Kenyatta National Hospital found that the antibiotics were mostly used for wound infections: flucloxacillin, gentamicin, ceftriaxone, cefuroxime, augmentin, ciprofloxacin, ceftazidime, cloxacillin, anti-tuberculous drugs, chloramphenicol and erythromycin (10)

1.8 Statement of the problem

Staphylococcus aureus is etiologic agents of a wide range of diseases, from minor infection to life-threatening invasive diseases. MRSA caused more than 19,000 deaths and 278,000 hospitalizations in the United States in 2005. In 2011, 80,461 severe MRSA infections occurred in the US Since the first outbreak of MRSA infection in Europe in the 1960s, severe S. aureus infections have become more prevalent, and transmission within health care and community have become more prevalent and transmission within health care and community settings has been a well-documented global health treat (1,36).

MRSA has become an endemic hospital pathogen in many countries. The US Centers for Disease Control and Prevention (CDC) report that MRSA infections now account for 63% of staphylococcal infections in the USA, after increasing from 2% in 1974 and 22% in 1995. In the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.), in which data were, collected from 33 centers in 11 Latin American countries (Argentina, Brazil, Chile, Colombia, Guatemala, Honduras, Jamaica, Mexico, Panama, Puerto Rico and Venezuela), the overall prevalence of MRSA (including HA- and CA-MRSA strains) among S. aureus isolates was 48.3% in 2004 - 2007. The SENTRY Antimicrobial Surveillance Program in Latin America revealed an increase in the prevalence of MRSA among staphylococcal infections in medical centers from 33.8% in 1997 to 40.2% in 2006, although these data are heavily weighted towards specific countries, with 41% of MRSA strains collected coming from Brazil**.** A number of other studies report nosocomial MRSA prevalence within Latin American countries, and these data point to differences in the pattern of resistance across the region. In a recent study, the prevalence of MRSA among S. aureus isolates from tertiary hospitals in Colombia, Ecuador, Peru, and Venezuelawas found to be 45%, 28%, 62% and 26%, respectively. Hospitalization, residence in long-term care facilities, surgery, hemodialysis, and contact with a person who has an MRSA infection, are all known risk factors for exposure to MRSA. 116 MRSA infections were first reported in hospitals with high levels of oxacillin or methicillin use, and nosocomial MRSA now tends to be multidrug resistant. Healthcare-acquired MRSA is typically defined by an MRSA infection that occurs more than 48 hours following exposure to a healthcare setting, although a precise designation of an infecting strain is only available through diagnostic testing (42).

S. *aureus* is a major health problem recognized as the most important nosocomial pathogen often causing respiratory tract infection, gastrointestinal infection, urinary tract infection, surgical site infection, blood stream infection and soft tissue infection. A study conducted from December 1, 2011 to March 30, 2012 among patients with surgical site infections at Debre Markos Referral Hospital, in Ethiopia showed that wound swabs obtained from patients with surgical site infections during the study period were isolated strains of S. *aureus* were tested for antibiotic susceptibility patterns using standard disc diffusion technique. S. *aureus* was isolated from 73 (39.7%) cases. Out of the 73 isolates of S. *aureus*, 36 (49.7%) were MRSA. Among the study participants, prevalence of MRSA was found to be 19.6%. The clinical isolates showed >80% level of resistance to ampicillin, amoxicillin, penicillin G, erythromycin, gentamicin and cotrimoxazole whereas <50% level of resistance was observed against clindamycin, oxacillin, tetracycline and vancomycin. MRSA strains showed resistance ranging from 5.6% (vancomycin) to 100% (cotrimoxazole). Of the following risk factors: sex, age, pus consistency, duration of operation, type of surgery, ward and hospital stay, laparotomy type of surgery was identified as a risk factor for infection by S. *aureus* (34).

In the study hospital culture and drug susceptibility test is commonly practiced but the common problems are clinicians start patient treatment with broad spectrum drugs before susceptibility test result delivered and the other problem is service interruption due to budget constraint. It demands attention from health practitioners as it results in mortality, morbidity and affects the wellbeing of patients. Most published studies of Hospital Acquired Infections (HAI) originate from hospitals in developed nations. Relatively, few data are available from Ethiopia to indicate the present HAI status (37).

In the study setting, there are limited data to show the recent the burden of MRSA in the TikurAnbessa Specialized Hospital and in general in Ethiopia. Hence, updating the information using the recent correctly collected data is crucial to fill the existing gap and design appropriate intervention methods.

1.9 Significance of the study

The findings of the study will to help in choosing appropriate antibiotics by considering the sensitivity patterns observed during the research experiment, hence appropriate management of the infection. This will result in a cost effective therapy for the patients and reduced financial burden of hospitalization. The Study will help, in identifying patients with MRSA infection and taking appropriate treatment after testing drug susceptibility test, in increasing the awareness of health professionals, patients and enhanced the prevention of the disease in the hospital, in assisting hospitals in planning hospital antibiotic policy and physicians will give due attention to diagnostic and microbiological analysis with cultures and antimicrobial sensitivities for the selection of a specific antimicrobial therapy. In addition, as far as my literature review goes, there is no published research from Ethiopia in my study setting; thus, the study findings can be used as base line data for further studies.

2. LITERATURE REVIEW

2.1 Prevalence of methicillin-resistant Staphylococcus aureus in Europe

In Europe the surveillance of bacteremia caused by methicillin-resistant Staphylococcus aureus (MRSA) is shown to be a problem affecting all European countries, although there is marked geographical variation in prevalence. Although the proportion of S. aureus bacteremia due to MRSA is declining in many countries, data from the European Antimicrobial Resistance Surveillance System (EARSS) for 2008 showed that in more than one third of countries, the proportion remained >25%. In contrast to bacteremia, community-associated MRSA infection in Europe remains relatively uncommon. However, there appears to be an increasing problem involving transmission of MRSA (particularly sequence type 398) from colonized livestock, particularly pigs, to farm workers, abattoir workers and veterinarians who are in contact with such animals. Molecular analysis of isolates of MRSA has shown that there has been spread of only a limited number of MRSA clones in Europe and that many of these clones show geographical clustering due to dissemination through regional healthcare networks. Despite our increasing understanding of the epidemiology of MRSA in Europe, MRSA infections continue to pose a significant public health challenge (2, 4 &9).

In England, surveillance of surgical site infections has been running since 1997. During the 5 years' period between January 2003 and December 2007, at least one causative microorganism was reported for 77% of surgical site infections. The most common organism was S. aureus (accounting for 38% of surgical site infections), of which 64% were MRSA.However, between October 2008 and September 2009, the proportion of S. aureus isolates (accounting for 31% of surgical site infections) that were methicillin resistant decreased to 32%.This decrease in surgical site infections due to MRSA in England appears to mirror the decline in MRSA bacteremia (66&67).

A cross sectional study conducted in Germany in patients in long-term care in hospitals, rehabilitation centers and nursing homes of a rural district S. aureus was isolated from 319 of 1 083 patients (614 females, 469 males; median age 68,2 years) from five hospitals, five nursing homes und three rehabilitation centers. The MRSA prevalence was 2.5% (95% confidence interval (CI) 1,7-3,7%). In hospitals the MRSA prevalence was 3.4%, in the nursing homes 2.3%

and in rehabilitation centers 1.2%, without any significant difference between these three establishments. The proportion of MRSA in all S. aureus isolates was 8.5% (33).

Another cross sectional study conducted in Italy University of Catania on 785 admitted pediatrics patients, affected with both acute and chronic diseases. MRSA nasal colonization prevalence among the admitted pediatrics was 1.15% (CI: 0.5607%-2.093%). Methicillin-sensitive Staphylococcus *aureus* (MSSA) nasal colonization prevalence at admission was 19.75% (CI 17.07%-22.64%). Five out of 9 MRSA-colonized patients had an underlying condition. Antibiotic therapy in the previous 6 months was a protective factor for both MRSA (OR 0,66; 95% CI: 0,46-0,96) and MSSA (OR 0,65; 95% CI: 0,45-0,97) colonization. A tendency to statistical significance was seen in the association between hospitalization in the 6 months prior to admission and MRSA colonization at admission (OR 4.92; 95% CI: 0,97-24,83). No patient was diagnosed with an *S. aureus* infection during hospitalization (34).

Another cross-sectional study conducted in Swedish hematological patients with febrile neutropenia. A routine blood cultures from febrile episodes occurring in adult patients with hematological disorders and neutropenia presenting to Karolinska University Hospital, Stockholm, Sweden during a 24-month period, were analyzed. *The culture result shows* from a total of 142 febrile neutropenic episodes occurring in, 124 hematological patients were included in the study. Bacteremia was documented in 27% of the episodes, and of these, 58% were due to Gram-positive pathogens. The most common isolates were viridans streptococci, coagulase-negative staphylococci, and Escherichia coli. Low levels of antibiotic resistance were detected. The underlying diagnosis of non-Hodgkin's lymphoma (NHL) was independently negatively associated with documented bacteremia ($p< 0.01$). From this the investigator concluded that the prevalence of bacteremia and the bacterial spectrum were consistent with recent Scandinavian reports. Substantially lower levels of antimicrobial resistance were registered compared to those found in other European centers. Patients with NHL were less likely to have documented bacteremia in this study (32).

2.2 MRSA Prevalence in Hospital Setting in Latin America Countries

MRSA has become an endemic hospital pathogen in many countries. The US Centers for Disease Control and Prevention (CDC) report that MRSA infections now account for 63% of staphylococcal infections in the USA, after increasing from 2% in 1974 and 22% in 1995.In the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.), in which data were collected from 33 centers in 11 Latin American countries (Argentina, Brazil, Chile, Colombia, Guatemala, Honduras, Jamaica, Mexico, Panama, Puerto Rico and Venezuela), the overall prevalence of MRSA (including HA- and CA-MRSA strains) among *S. aureus* isolates was 48.3% in 2004-2007. The SENTRY Antimicrobial Surveillance Program in Latin America revealed an increase in the prevalence of MRSA among staphylococcal infections in medical centers from 33.8% in 1997 to 40.2% in 2006, although these data are heavily weighted towards specific countries, with 41% of MRSA strains collected coming from Brazil. A number of other studies report nosocomial MRSA prevalence within Latin American countries, and these data point to differences in the pattern of resistance across the region. In a recent study, the prevalence of MRSA among *S. aureus* isolates from tertiary hospitals in Colombia, Ecuador, Peru, and Venezuela was found to be 45%, 28%, 62% and 26%, respectively (42).

Hospitalization, residence in long-term care facilities, surgery, hemodialysis, and contact with a person who has an MRSA infection, are all known risk factors for exposure to MRSA. MRSA infections were first reported in hospitals with high levels of oxacillin or methicillin use, and nosocomial MRSA now tends to be multidrug resistant. Healthcare-acquired MRSA is typically defined by an MRSA infection that occurs more than 48 hours following exposure to a healthcare setting, although a precise designation of an infecting strain is only available through diagnostic testing (42).

2.3 Prevalence of MRSA in some Assian Countries

A study conducted in India reveals that a total of 100 S. aureus isolates were tested for oxacillin (methicillin) resistance as well as vancomycin resistance respectively. Of the 100 tested, 63 were from male and 37 from female patients. Of the total 100 S. aureus isolates 52 were detected as MRSA and the other 48 as MSSA. Of the 52 MRSA isolates 32 (61.54%) were from male and 20 (38.46%) from female patients. Thus maximum i.e. 36.54% MRSA were isolated from blood

samples i.e. patients with suspected/diagnosed sepsis; followed by 28.85% from pus i.e. skin and soft tissue infections, 15.38% from urine – urinary tract infections and 11.54% from sputum samples representing respiratory tract infections and least from body fluids and E.T tips (endotracheal tips) each 3.85%. Maximum numbers of MRSA isolates were obtained from samples of ICU i.e. 38.46%, followed by 15.38% from Medicine, 11.54% Surgery, Orthopedics 9.62% and minimum from Plastic Surgery Ward (1.92%). Erythromycin induced resistance against clindamycin was seen in only 13 out of MRSA(n=52) strains and 4 out of MSSA strains (n=48) (25)

Another study conducted in the same country India shows that a total of 291 clinical specimens of S. aureus that were subjected to MRSA screening were collected from 156 males and 135 females. The MRSA positivity among males was significantly higher than females' counterparts. Extremely significant higher MRSA positive cases was observed from ages less than 30 years, with stay of more than 15 days, and previous history of intake of broad spectrum of antibiotics. Incidentally, there was no significant difference of MRSA positivity with a previous history of hospitalization in their study (26.)

A study conducted in Bangladesh Isolation rate of Staph aureus among admitted patients from nasal swab sample were recorded. After screening 500 nasal swabs, 255(51%) isolates were culture positive for Staphylococcus. Out of 255 Staphylococcus, 112 (22.4%) were Staph aureus and 143 (28.6%) were coagulase negative Staphylococcus isolation rate of MRSA and MSSA among Staphylococcus aureus was recorded. Out of 112 Staph aureus,38 (33.9%) strains were detected as MRSA and 74 (66.1%) strains were detected as MSSA (27).

Another related study conducted in Bangladesh showed that MRSA is a causative agent of hospital-acquired infection and an incipient community pathogen in many geographical regions. In the present study, the isolation rate of S. aureus (72.5%) from burn wound patients was high, as the microorganism was confirmed in 29 of the 40 isolated strains of Staphylococcus species based on cultural, biochemical, and coagulase properties. In addition, imipenem was found to be the most effective antibiotic against the isolates with 90% of strains exhibiting sensitivity to this drug. Most of the isolates (80%) were also sensitive to netilmicin, clindamycin, and nitrofurantoin. Almost 55% of isolates were sensitive to amikacin, chloramphenicol, gentamycin, and to bramycin. However, 26 of 29 strains of S. aureus were resistant to penicillin

G and 75% of isolates were resistant to azithromycin, ciprofloxacin, methicillin, oxacillin, and tetracycline. Approximately 65% of isolates exhibited resistance to erythromycin, and trimethoprime sulfamethoxazole. Twenty-one of the 29 S. *aureus* isolates studied were found to be MRSA. The prevalence of methicillin resistance among staphylococci isolated from burn patients in our hospital has not been determined accurately to date. In this study, the prevalence of MRSA was 72%, which varied from findings in other studies in other countries (28).

A study conducted in Pakistan reveals that S. aureus were isolated from wound exudates of hospitalized patients and characterized by biochemical tests. Out of 200 samples, 110 isolates were presumably recognized as S. aureus on them being coagulase positive, which further were characterized upon their biochemical profile. The remaining isolates contain coagulase negative Staphylococcus (CONS) and based upon the absence of Mannitol fermentation character was considered as Staphylococcus epidermidis. It was observed that out of 200 samples, 110(55%) were coagulase positive S. *aureus* isolates, 51(25.5%) isolates were MRSA and remaining56 (50.92%) isolates were MSSA and 3 (2.73%) isolates were methicillin intermediate S. *aureus*. Prevalence of MRSA was recorded 18%, 38%, 22%, 24%, in Services Hospital, Mayo Hospital, Jinnah Hospital, Ganga Ram Hospital, respectively(29).

2.4 Prevalence of MRSA in some African Countries

A study conducted in Kenya reveals that a total of 107 isolates of S. aureus were obtained, of which 39% (37) were MRSA. Most of the MRSA, 13 (33%) and 7 (17%), were found in pus and tracheal aspirate samples respectively. Majority of the MRSA isolates were from surgical wards and ICU. The MRSA isolates were highly resistant to Erythromycin (92%;36/39), and tetracycline (92%;36/36) and moderately susceptible to linezolid (77%;30/39). A total of 28 (74.4%) MRSA isolates were Clindamycin inducible resistant (30).

A study conducted Among healthcare workers and students in Madagascar showed that of 1548 nasal swabs tested, 171 (11 %) were positive for S. aureus; 20 (1.3 %) of these isolates were identified as MRSA. S. aureus was detected in 91 of 863 healthcare workers (10.4 %) and in 80 (11.8 %) of 685 students; however, 14 (1.5 %) healthcare workers carried MRSA compared with six (0.9 %) students. Nasal carriage of S. aureus and MRSA was more prevalent in women than in men, and 21 (11.7 %) S. aureus isolates were PVL positive and 36 (21 %) were TSST-1 positive (44).

A study conducted in Dar Es Salaam, Tanzaniaon 169 patients and 47 health workers who were recruited, the mean age was 43.4 years ± SD 15.3 and 37.7 years ± (SD) 11.44 respectively. Among the patient's male contributed 108 (63.9%) while in health worker majority 39(83%) were females. The prevalence of MRSA colonization among patients and health care workers was 11.83% and 2.1% respectively. All (21) MRSA isolates were highly resistant to penicillin and erythromycin, and 17 (85.7%) were highly sensitive to vancomycin. Being male (AOR 6.74, 95% CI 1.31-34.76), history of sickness in past year (AOR 4.89, 95% CI 1.82- 13.12), being sick for more 3 times (AOR 8.91, 95% CI 2.32-34.20), being diabetic (AOR 4.87, 95% CI 1.55-15.36) and illicit drug use (AOR 10.18, 95%CI 1.36-76.52) were found to be independently associated with MRSA colonization (45).

Another cross sectional study conducted in Asmara, Eritreaon 130 study participants recruited from two referral hospitals. Among the study participants the prevalence of coagulase positive S. aureus was 82(63.1%). The prevalence of MRSA among the isolates was 59(72%). The isolation was mostly from the pus specimen in burn, diabetics, and surgical wound patients. Patients <18 years of age were more likely to be colonized by S. aureus compared to patients above 61 years. The isolates showed 13(15.9%) of resistance to vancomycin, 9(11%) to erythromycin, and 1(1.2%) to gentamycin (61).

2.5 MRSA Surveillance in Ethiopia

A study conducted in Ethiopia, Mekele showed that a total of 249 HIV positive individuals attending HIV care service in four health care facilities were included in the study. The majority of the participants were females 174 (69.9 %). The age ranges of participants were 5–72 years with a mean age of 35 years. The majority 103 (41.4 %) of the participants were in the age group of 30–39. The overall rate of S. aureus and MRSA colonization among the 249 study participants were 81 (32.5 %) and 6 (2.4 %) respectively. Distribution of MRSA by nasal, throat and both sites were 3, 2, 1 respectively and for S. aureus was 41 (50.6 %), 28 (34.6 %) and 12 (14.8 %) respectively **(**31**).**

A study conducted in Ethiopia,TikurAnbessa hospital, on a total of 188 Swabs from post-surgery wound infection, ear infection and corresponding nasal swabs were collected using convenient sampling method. Samples were cultured for S. aureus according to standard procedures.

Isolates were tested for susceptibility to panels of 18 antimicrobial agents using disc diffusion assay. Susceptibility to methicillin was phenotypically determined based on sensitivity of isolates to cefoxitin and oxacillin. A total of 79(57.4%) S. aureus isolates were recovered. 40 isolates were from wound swabs and 39 from nasal swab. The isolates were resistant to Ampicillin (100%), Oxacillin and Cefoxitin (68.4%, each), Clindamycin (63.3%), Cephalothin (59.5%), Tetracycline (57%), Cotrimoxazole and Bacitracin (53.2%, each), and Erythromycin (51.9%). Majority of the isolates (n=67, 94.9%) exhibited multi drug resistance and one isolate was resistant to all the tested drugs. 68% of the isolates were methicillin resistant (MRSA) and 44.3% were resistant for vancomycin. In conclusion, S. aureus isolates from patients in TikurAnbessa specialized hospital exhibited resistance to antibiotics most commonly used for the treatment of staphylococcal infections (43).

Another cross-sectional study which was conducted from December 1, 2011 to March 30, 2012 among patients with surgical site infections at Debre Markos Referral Hospital in Ethiopia. Wound swabs obtained from patients with surgical site infections during the study period were cultured on mannitol salt agar media which is selective for S. *aureus*. Isolated strains of S. *aureus* were tested for antibiotic susceptibility patterns using standard disc diffusion technique, and interpretation of resistance was done based on Clinical and Laboratory Standard Institute criteria. Univariate and multivariable analyses were used to assess the risk factors. This study carried out on 184 surgical patients who had developed surgical site infection. S. *aureus* was isolated from 73 (39.7%) cases. Out of the 73 isolates of S. aureus, 36 (49.7%) were MRSA. Among the study participants, prevalence of MRSA was found to be 19.6%. The clinical isolates showed >80% level of resistance to ampicillin, amoxicillin, penicillin G, erythromycin, gentamicin and cotrimoxazole whereas <50% level of resistance was observed against clindamycin, oxacillin, tetracycline and vancomycin. MRSA strains showed resistance ranging from 5.6% (vancomycin) to 100% (cotrimoxazole). Of the following risk factors: sex, age, pus consistency, duration of operation, type of surgery, ward and hospital stay, laparotomy type of surgery was identified as a risk factor for infection by S. *aureus* (34).

A cross sectional study was conducted among 1360 participants at Yekatit 12 Hospital Medical College in Ethiopia from September 2013 to April 2014. Clinical samples from various anatomical sites of study participants were cultured on blood agar and mannitol salt agar and

identified to be S. aureus by using catalase, coagulase and DNAse tests. S. aureus isolates then were screened for MRSA using 30 µgcefoxitin disc and other 11 antimicrobial drugs by disc diffusion procedure, and agar dilution and E tests for vancomycin. Of 1360 clinical specimens analyzed S. aureus was recovered from (194, 14.3 %). Rate of isolation of S. aureus with regard to clinical specimens was the highest in pus (118, 55.4 %). No S. aureus was isolated from CSF and urethral discharge. Out of 194 S. aureus isolates, (34, 17.5 %) were found out to be MRSA and the remaining (160, 82.5 %) were MSSA. Ninety-eight (50.5 %) S. aureus were multi drug resistant and the highest isolates were resistant to penicillin (187, 96.4 %) and least resistant for clindamycin (23, 11.9 %) and vancomycin (10, 5.1 %). MRSA strains were 100 % resistant to penicillin G, erythromycin, trimethoprim-sulfamethoxazole and least resistant to vancomycin (10, 29.4 %). Out of 194 S. aureus isolates (153, 79.0 %) were beta-lactamase producers (35).

2.7 Factors influencing the development of nosocomial infections

2.7.1 The microbial agent

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not necessarily result in the development of clinical disease. Other factors also influence the nature and frequency of nosocomial infections. The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (inoculum) of infective material. Many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patient ‘s own flora (endogenous infection). Some organisms may be acquired from an inanimate object or substances recently contaminated from another human source (environmental infection) (1,39 &40).

Before the introduction of basic hygienic practices and antibiotics into medical practice, most hospital infections were due to pathogens of external origin (foodborne and airborne diseases, gas gangrene, tetanus, etc.) or were caused by microorganisms not present in the normal flora of the patients (e.g. diphtheria, tuberculosis). Progress in the antibiotic treatment of bacterial infections has considerably reduced mortality from many infectious diseases. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients

(*Staphylococcusaureus*, coagulase-negative staphylococci, enterococci, Enterobacteriaceae) (13, 39).

2.7.2. Patient susceptibility

Important patient factors influencing acquisition of infection include age, immune status, underlying disease, and diagnostic and therapeutic interventions.

The extremes of life (infancy and old age) are associated with an inadequate/decreased resistance to infection. Patients with chronic disease such as malignant tumours, leukaemia, diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome (AIDS) also have an increased susceptibility to infections with opportunistic pathogens. The latter are infections with organism(s) that are normally innocuous, e.g. part of the normal bacterial flora in the human, but may become pathogenic when the body's immunological defenses are compromised. Immunosuppressive drugs or irradiation may also lower resistance to infection. Injuries to skin or mucous membranes bypasses natural defense mechanisms poses risk of infections (13,40).

Malnutrition is also a risk factor. Many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, intubation/ventilation and suction and surgical procedures increase the risk of infection. Contaminated objects or substances may be introduced directly into tissues or normally sterile sites such as the urinary tract and the lower respiratory tract (19, 40).

2.7.3 Environmental factors

Health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for other patients and staff. Patients who become infected in the hospital are a further source of infection. Crowded conditions within the hospital, frequent transfers of patients from one unit to another, and concentration of patients 32 highly susceptible to infection in one area (e.g. newborn infants, burn patients, and intensive care) all contribute to the development of nosocomial infections. Microbial flora may contaminate objects, devices, and materials used in one procedure which subsequently contact susceptible body sites of other patients. In addition, new infections associated with bacteria such as waterborne bacteria (atypical mycobacteria) and/or viruses and parasites continue to be identified (41).

2.7.4 Bacterial resistance

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements, antibiotics promote the emergence of multiple antibiotic resistant strains of microbes; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. The widespread use of antimicrobials for therapy or prophylaxis is the major stimulant of resistance. Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial agent becomes widely used, bacteria resistant to this drug eventually emerge and may spread in the health care setting. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective. Multiple resistant *Klebsiella*and *Pseudomonasaeruginosa*are prevalent in many hospitals. This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable (38).

3. Objectives

3.1 General Objective

To assess the Prevalence and Anti-microbial susceptibility patterns of MRSA and MSSA among patients suspected for HAI in admitted patients and associated risk factors in Black Lion Tertiary Care Hospital (ICU, Gynecology, Hematology, Pediatrics, Orthopedics & Surgery).

3.2 Specific Objectives

To determine the prevalence of MRSA and MSSA in Black Lion Tertiary Care Hospital.

To assess the Anti-microbial susceptibility patterns of MRSA.

To assess the association risk factors with the acquisitions MRSA and MSSA.

4. Hypothesis

There is no association between duration of hospital admission length in days and acquisition of MRSA where P-values < 0.05.

There is no association between admission wards and acquisition of MRSA where P-values <0.05.

There is no association between sex and acquisition of MRSA where P-values <0.05.

There is no association between age and acquisition of MRSA where P- values <0.05.

There is no association between blood transfusion and acquisition of MRSA where- P values <0.05.

There is no association between the sample type used (pus and blood) and acquisition of MRSA.

There is no association between patients who has taken Antibiotics within the last six months and acquisition of MRSA.

5. Materials and Methods

5.1 Study area

The study carried out at Addis Ababa University Collage of Health Sciences TikurAnbessa Specialized hospital, in Lideta sub-city, Addis Ababa, Ethiopia. The hospital has 800 beds. It provides services for the community in and outside the capital as referral services such as OPD, surgery, family planning, ART, laboratory, pharmacy, ANC, Oncology, ENT, delivery and neonatal careand etc.

5.2 Study Design and Period

A cross sectional study conducted among 413 study participants who are suspected for hospital acquired infections at Addis Ababa University Collage of Health Science TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia from January 2018 to January 2019.

5.3 Population

5.3.1 Source Population

All patients admitted in TikurAnbessa Specialized Hospital during the study period.

5.3.2 Target Population

All patients attending *ICU, surgery, Hematology, Pediatrics, Orthopedics* and *gynecology* wards who were suspected by the physician to bacterial nosocomial infection after 48 hours of admission or patients with sign and symptom of bacterial nosocomial infection in TikurAnbessaSpecialisedhospital during the study period were the target population.

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion criteria

All patients attending ICU, Surgery, Hematology, Pediatrics, Orthopedics and Gynecology with sign and symptom of bacterial nosocomial infections (WHO, 2002) were included.

5.4.2 Exclusion criteria

All patients those did not consent to participate in the studywere excluded**.**

All patients out of ICU, Gynecology, Hematology, Pediatrics, Orthopedics and surgery were excluded.

All patients without sign and symptom of bacterial nosocomial infections after 48 hours were excluded (WHO, 2002).

5.5 Study variables

5.5.1 Dependent variable

- Antimicrobial sensitivity patterns of the S. au*reus.*
- Prevalence of MRSA and MSSA.

5.5.2 Independent variable

- age, sex, hospital staying duration, drugs taken, and wards.

5.6 Sample size calculation and Sampling method

5.6.1 Sample size calculation

The sample size is determined using single population proportion formula considering the 95% confidence level taking the prevalence as 30.9% since there is a similar study conducted in South African five Tertiary Hospitals in 2017(41) and Degree of allowable error 0.05. There is no similar study conducted before in Ethiopia to show the overall burden of MRSA among admitted patients in the hospital. Therefore, I used the data from the South African. The sample size is therefore calculated as follows:

Total study subjects**:** $\mathbf{n = z^2\, p\, (1\text{-}p)}$ =364

$$\frac{}{\mathbf{d2}}$$

Where:

n= sample size

Z= Standard normal deviate at 95% confidence interval.

P= Proportion of target population infected with MRSA taken 30.9% from previous similar study in South Africa tertiary care hospitals.

q= 1-p

d= degree of freedom.

Z= 1.96, p=30.9% q=69.1% d=0.05

Thus;

n= $1.96^2 x 0.309 x 0.691 / 0.05^2$

=328

Including 10% non-respondent rate, the total sample size will be

n= 328/0.9= 364

5.6.2 Sampling Method

A total of 413study participants were recruited using convenient sampling technique who show sign and symptoms of nosocomial infection (WHO, 2002) during the study period**.**

5.7. Measurement and Data collection

5.7.1. Data collection procedure

Permission was given from the consultant in charge of the ward before commencing on data collection. After providing informed consent, demographic data (e.g., sex, age, ….) were collected via a questionnaire (Appendix 1). Respondents were asked about hospital admission within the last 6 months, their history of antimicrobial treatment within the last 6 months (1), whether they suffer from a chronic illness, and whether they had any acute skin diseases such as atopic dermatitis, psoriasis or chronic ulcers. As S. aurous colonizes pets and livestock, the participants were asked whether they had contact with animals. After the participants filled in the questionnaire, clinical samples were collected by the nurses and the principal investigator.

The principal investigator demonstrated the technique used to obtain clinical samples with a sterile and provide assistance to the nurses where necessary. The samples then immediately transported to the study laboratory where the bacteria were isolated as described below. After incubation on blood agar for 18–24 h at 35–37 °C, the first step of the identification procedure involved examination of colony morphology and Gram staining. Suspected S. Aureus colonies on the blood agar plate were tested for catalase and coagulase activity, and for latex agglutination. S. aureus strains were tested for antibiotic susceptibility using the standard disc

diffusion method on Mueller-Hinton Agar, according to current CLSI, 2018 (Clinical and Laboratory Standards Institute) guidelines, 2018. The antibiotics that are test include; amoxicillin, clavulanic acid, cefuroxime, ceftriaxone, imipenem, ciprofloxacin, cefoxitin, cloxacillin and ceftazidime. All data is kept under lock and key, with accessibility limited to the researcher only.

5.7.3 Laboratory Analysis

5.7.3 .1. Sample collection and Examination

Clinical samples were collected from patients admitted in *ICU,Gynecology, Hematology, pediatrics, Orthopedics* and *surgery*who develop infection after 48 hours by employing standard microbiological procedures. Pus from wound and blood were the clinical specimens collected. All specimens were transported to microbiology laboratory of the hospital with minimum delayaccording to (CLSI,2018) for culture and sensitivity tests. Clinical specimens were inoculated onto 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 °C for 18 to 24 h 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 gram stain and biochemical tests. Catalase positive, coagulase positive, postrex staph extract and gram positive bacteria appearing in grape like cluster were considered S. aureus.

5.7.3.2 Blood sampling and processing

About 2.5-5ml of blood specimen Inoculated into aerobic 30ml BACT/ALERT PF Plus bottles at the blood to broth ratio of 1: 10-1:30 and two bottles of blood samples are collected per patient and incubated aerobically at 37 0C for 5 days. The incubation was followed strictly for bacterial growth alarm (Annex I).

5.7.3. 3. Pus from wound infection

Wound specimens were collected from appropriate site of infection aseptically using sterile swab. It was cultured on both Mannitol salt agar and blood agar. Followed by gram stain then biochemical test were conducted in order to isolate the causative agents. Detail procedure is attached in the annex (Annex I).

5.7.3.4 Culture and Gram staining

Clinical specimens were inoculated onto blood agar plate (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 °C for 18 to 24 h 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, biochemical tests, catalase coagulase and postrex staph extract. Those Catalase, coagulase, postrex staph extracts and gram positive bacteria appearing in grape like cluster will be considered as Staphylococcus aurous (Annex I).

5.7.3.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility test was carried out by Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI, 2018) guidelines on Muller Hinton agar (Oxoid, Basingstoke, England) for 10 anti-microbials. 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 1 × 106 colony forming units (CFU) per ml. A sterile swab was dipped into the suspension and the excess of inoculums was removed by pressing it against the sides of the tube. Then the swab was applied to the center of Muller Hinton agar plate and evenly spread on the medium. Antibiotic discs were placed after 15 min of inoculation to Muller Hinton agar seeded with each isolate and were incubated for 24 h at 35–37 °C. The diameter of the zone of inhibition around the disc was measured using sliding metal caliper (12).

5.8 Data quality Assurance

Data collection sheets were checked for their completeness, readability and clearness using pre-testing these were delivered for 5% of participants. Socio-demographic characteristics of the patient were collected appropriately using data collection sheets. Samples were collected in accordance with SOPs and were sent as soon as to the laboratory with a minimum delay(CLSI,2018) for analysis. Culture results were recorded carefully before entry to statistical tool. Then data were double checked and entered with both SPSS 24.

All specimens were collected according to the standard operating procedure to ensure the accuracy of data, materials and equipment used. Performance of the equipment were checked by

pilot test, using standards and daily quality control. Standard Operating Procedures (SOP) were strictly followed verifying that media meet expiration date and quality control parameters per CLSI standards. Visual inspections of cracks in media or plastic Petri dishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. QC was performed to check the quality of medium. Each new lot was quality controlled before use for testing the *Staphylococcus aurous using* standard strains *(ATCC25923).*

5.9 Statistical analysis

Data was entered and analyzed using SPSS software version 24 (SPSS INC, Chicago, IL, USA). Chi square test (Binary and Multivariate logistic regression) were used to determine the association between MRSA Acquisition and hospital stay length, age, sex, blood transfusion, specimen type previous admission history and drugs used. The analysis was used to see the relation between dependent variable and independent variables. Finally, the results were presented in words, charts, graphs and tables. The descriptive statistics (mean, percentages or frequency) were calculated and used to summarize the collected data. P-values less < 0.05 was taken as statistically significant.

5.10 Ethical considerations

The study was approved by "Department Research and Ethical Review Committee of the Department of Medical Laboratory Science, School of Health Sciences, College of Health Sciences, Addis Ababa University. Permission letter was also obtained from the study site. The purpose and procedures of the study was explained to the study participants within the study period. Those patients who were given informed consent were selected and enrolled as the participants of the study. The consent of very critical patients (unable to respond) were obtained from their family. All the information obtained from study participants were kept confidential and the specimen collected from the patients were used only for this study purpose. There was not any direct payment for participating in the study. But any positive finding in laboratory examination result were reported to their physician for appropriate treatment and management.

5.11. Dissemination of the result

The results of the study were submitted to the Department of Medical Laboratory Sciences, School of Health Sciences, College of Health Sciences, and Addis Ababa University. In addition, the results were submitted to the study site. Abstract were submitted (like EMA, EPHA and

EMLA) and other international associations to present the results during continuous medical education events or conference organized by these associations.

5.12 Operational definition

MRSA is defined as zone of inhibition less than or equal to 21 mm on MHA with 30 µgcefoxitin/Oxacillindisc seeded with growth suspension of S. aureus isolates adjusted to 0.5 McFarland standards (12).

Invasive procedure refers to a diagnostic or therapeutic technique that requires skin penetration or entry of a body cavity.

MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories.

Nosocomial infection refers to an infection developed from hospital environment after 48 hours' admission or infections acquired during hospital stay which are not present during admission (WHO,2002).

Risk factor refers to any attribute, characteristic or exposures that increases or decrease the likelihood of developing a disease**.**

Intensive care unit also known as an intensive treatment unit refers to a special department of a hospital or health care facility that provides intensive care medicine**.**

Device Associated Infection refers to an infection which comes after a patient exposed to materials support for the patient body function.

Duration of admission is defined as total number of days or weeks a patient staying in the hospital.

6. Results

6.1. General Description

A total of 413 samples were collected from 413 patients suspected for hospital acquired infections. The clinical specimen collected from the patients were blood and pus samples. Patients admitted in Gynecology ward, orthopedic ward, general surgical ward, ICU ward, hematology ward and pediatrics ward who showed sign and symptoms of hospital acquired infection were included. In this study 190 female patients and 223 male patients who has been suspected for hospital acquired infection has been involved. The study participants age ranges from 1 to 75 years. The mean age of the study participants were 28 years. MRSA prevalence was highest in the age groups (30-49) and its burden was (21,5.1%) and the least was observed in the age groups >70 and the burden was (0.0, 0.0%). In the study participants, the overall prevalence of Coagulase Positive *S. aureus* was 38.7% (160/413). The prevalence of *MRSA in wards from*

the isolates were 16/57(28.1%), 15/57(26.3%), 10/57(17.5%), 8/57(14%), 4/57(7%), 4/57(7%) in hematology, surgery, ICU, pediatrics, orthopedics, and gynecology respectively. From the total isolates identified, *MRSA* was highest in Hematology wards 28.1%(16/57) and the least was observed in Orthopedics and Gynecology, each accounts 7%(4/57). The prevalence of MRSA in blood stream accounted 45.6%(26/57) and in pus sample is 54.4%(31/57). The overall prevalence of MRSA in the study population was 13.8%(57/413).

The Prevalence of S. *aureus* in males and females were 55.6% (89/160) and 44.4% (71/160) respectively. The highest *MRSA* burden were found in the age groups (31-40) which was found to be (22.8%, 13/57) (Table-1 and Table-2).

In our study population, MRSA was highest in Hematology wards (16/57, 28.1%) followed by surgery wards (15/57,26.3%). It is found that the MRSA burden in the isolates from females' population were (38.6%, 22/57) less than males (61.4%, 35/57) and from the isolates the prevalence of MRSA was highest in the age ranges (31-40) and their prevalence were (22.8%,13/57) and the least was observed in the age ranges (>70)and its prevalence was (1.8%,1/57) (Table-5) and (figure-1).

In our study, patients who has previous history of admission with in the last six months acquired higher MRSA 40(70.2%) than patients who has no previous history of admission. And the rest 17(29.8%) MRSA were found in patients who has no previous history of admission. Although the occurrence MRSA acquisition is higher among patients who have previous history of admission, but it was not statisticallysignificant(where P value <0.301) (Table-1).

Table-1: The prevalence of MRSA among patients who has previous history of admission, admitted in between January 2018 up January 2019 (n=57).

Previous admission history	MRSA	MRSA%	MSSA	MSSA%	P value
Yes	40	70.2%	75	72.8%	
No	17	29.8%	28	27.2%	P<0.301
Yes + No	57	100%	103	100%	

Patients who has Previous history of medication conception with in the last six months' periods among admitted patients in different wards, in the hospital showed resistance to a variety of drugs tested including cefoxitin hence it contributed a lot to acquire a significant amount of MRSA in the patients. Therefore, acquisition of MRSA is strongly associated with previous conception of drugs where P value <0.001 (Table -2).

Table 2: The prevalence of *MRSA and MSSA*among patients who has previous medication conception history within the last six months in between January 2018 up to January 2019(n=160).

Previous history of Antibiotic usage within the last six months.	Response	MRSA	MSSA	P value
	Yes	51	81	P<0.001
	No	6	22	
	Yes +No	57	103	

Most of the MRSA were recovered from pus sample than blood samples. (31, 54.4%) MRSA recovered from pus and (26, 45.6%) blood samples respectively. As compared to blood samples, recovering of MRSA was higher in pus samples than blood, hence it was statistically significant (where P value<0.0) (Table-3).

Table-3: Distribution of Staphylococcus aureus (MSSA) and MRSA isolates in pus and blood samples in admitted patients in between January 2018 upto January 2019 (n=160)

Specimen type	MRSA(%)	MSSA(%)	Total(%)	P value
Blood	26(16.3%)	42(26.2%)	68(42.5%)	P<0.0
Pus	31(19.4%)	61(38.1%)	92(57.5%)	
Blood + pus	57(35.7%)	103(64.3%)	160(100%)	

Based on the WHO 2018 age classification, MRSA distribution in different age groups had been stated below. The highest MRSA burden were found in the age groups (30-49) which was found

to be (5.1%, 21/413)followed by the age groups (15-29), 5-14 and <5. The burden in the age groups were (14, 3.4%), (6,1.5% in each) of the age groups 5-14 and <5. The least was observed in the age groups >70 and its burden was (0.0%) among the isolates. Acquisition of MRSA in the age groups were statistically significantwhere (P Value<0.0) (Table-4).

Table -4: Age based distribution characteristics of hospital acquired MRSA among admitted patients in between January 2018 up to January ,2019.(n=57/413)

Age Groups	MRSA Isolated	Percentages	P value
<5	6	1.5%	P<0.0
5-14	6	1.5%	
15-29	14	3.4%	
30-49	21	5.1%	
50-59	4	1.0%	
60-69	5	1.2%	
>70	1	0.2%	
Total	57	13.8%	

The overall burden among the patients, MRSA was revealed in 57/413 (13.8%), and the remaining 103/413 (25%) isolates were identified methicillin sensitive whereas 29/413(7.02%) are CONS (Table- 5).The Prevalence of S. *aureus* in males and females were 55.6% (89/160) and 44.4% (71/160) respectively. MRSA was highest among the isolates in males and its burden was (35,61.4%) where as in female it's burden was (22, 38.6%). Although the prevalence of MRSA was relatively higher in males' population, acquisition of MRSA in sex groups was not statistically significant where (P vale<0.4) (Table-5) and (Fig-1).

Table-5:Gender based Distribution of (MSSA) and MRSA isolates in between January 2018 up to January, 2019(n=160).

Sex	**MRSA**	**MSSA**	**P value**

Male	**35**	**54**	**P<0.4**
Female	**22**	**49**	
Total Count	**57**	**103**	

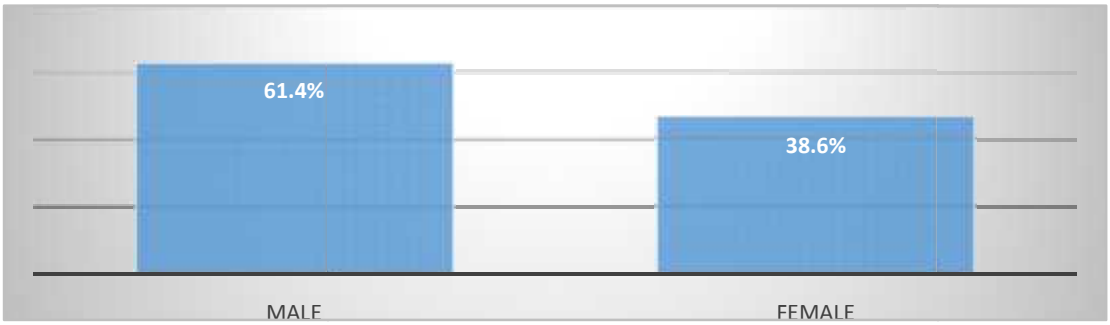


Figure-1: A graphical presentationof gender based distribution of MRSA among admitted patients in between January 2018 up to January, 2019 (n=57).

In the determination of the susceptibility of coagulase positive *S. aureus* on nine selected antibiotics by disk diffusion technique, the isolates were resistant to Penicillin 143(89.4%) followed by erythromycin 93(58.1%), gentamicin 87(54.4%%), Ciprofloxacin 89(55.6%), Cefoxitin and oxacillin 57(35.6%), Trimethoprim Sulfamethoxazole 69(43.1%), Clindamycin 9(5.6%), Augumentin, ceftriaxone, Cefepime, Cephalexin, Cefuroxime, Cefotaxime and Meropenemeach showed 57(35.6%)resistance respectively (Table-6).

Table-6: Antimicrobial susceptibility profile of *S. aureus*isolates among the admitted patients in between January 2018 up to January, 2019)(n=160).

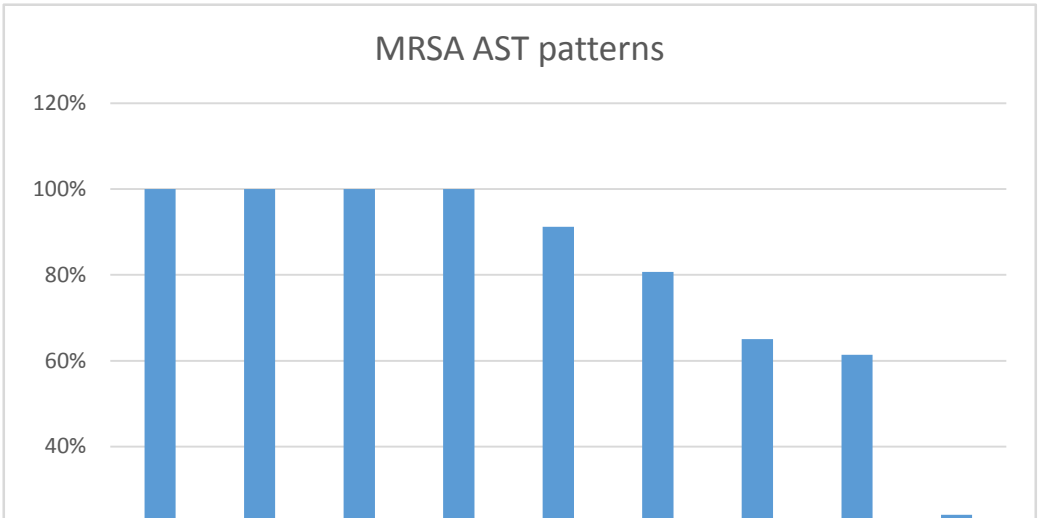
S.No	**Antimicrobial agents in (ug)**	**S**	**R**
1.	Erythromycin (15)	67(41.9%)	93(58.1%)
2.	Cefoxitin (30)	103(64.4%)	57(35.6%)
3.	Clindamayicin (2)	144(75.6%)	16(28.1%)
4.	Pencillin(10)	17(10.6%)	143(89.4%)
5.	Trimethoprim -Sulfamethoxazole	91(56.9%)	69(43.1%)
6.	Ceftriaxone (30	103(64.4%)	57(35.6%)

7.	Augumentin(30)	103(64.4%)	57(35.6%)
8.	Ciprofloxacin (5)	71(44.4%)	89(55.6%)
9.	Gentamyicin(10)	73(45.6%)	87(54.4%)
10.	Cefepime(30)	103(64.4%)	57(35.6%)
11.	Cephalexin (30)	103(64.4%)	57(35.6%)
12.	Cefuroxime (30)	103(64.4%)	57(35.6%)
13.	Cefotaxime (30)	103(64.4%)	57(35.6%)
14.	Meropenem(10)	103(64.4%)	57(35.6%)

S= Sensitive R= Resistance

In the determination of the susceptibility patterns of MRSA isolates, all 57 MRSA strains were 100% resistant to Penicillin, Augmentin, Cefoxitin and other Cephalosporins including cephims, in contrast Clindamycin (16, 28.1%), Trimethoprim sulfamexozole (37,65%), Erythromycin (35, 61.4%), Gentamycin (52, 91.2%), ciprofloxacin (46, 80.7%) resistant each respectively(fig-2).

Figure -2: A graphical presentationof antibacterial Susceptibility patterns of *MRSA*isolates in admitted patients in between January 2018 and January, 2019 (n=57).



ERY=Erythromycin Clin=Clindamycin PEN=Penicillin STX=Trimethoprim-Sulfometexazole, ABLs= Other Beta Lactams, Aug= Augmentin, Cipro= Ciprofloxacin, Gen= Gentamycin, Fox= Cefoxitin

The antimicrobial susceptibility pattern of MRSA isolates from different wards from different patients in the hospital were found to be highly variable. Among the isolates of MRSA, the highest isolates rate was seen in hematology wards which accounts (16,28.1%) and the least seen in Orthopedics and gynecology each accounts (4,7%) respectively. The risk factors for the patients in wards could be transfusion of blood and blood products routinely, lack of ventilation of the wards, shortageof water supply for personal hygiene and cleaning, occurrence of bed sore, chronic wounds, long term usage of vein for injection and blood transfusion, catheter usage and previous admission history contributed a lot for acquisition of MRSA.In general, all MRSA strains proved multidrug resistance in the wards. Hence, due to these various factors, acquisition of MRSA in the setting wards/departments was statistically highly significant (where P value=0.014) (Table-7 and Fig-2).

Table -7 Occurrence of Staphylococcus aureus (MSSA) and (MRS) isolates in admitted patient'srelation to hospital 's wards/departments in between January 2018 up to February,2019(n=160).

S. No	Wards	MSSA(%)	MRSA(%)	Total	P value
1	Orthopedics	30(29.1)	4(7)	34	
2	Gynecology	8(7.8)	4(7)	12	
3	Hematology	16(15.5)	16(28.1)	32	
4	Surgery	22(21.4)	15(26.3)	37	

5	Pediatrics	12(11.6)	8(14)	20	P<0.014
6	ICU	15(14.6)	10(17.5)	25	
Total Count		103	57	160	
Total%		64.4%	35.6%	100%	

In the acquisition of MRSA, longer duration of admission in the hospital was not contributed a significant MRSA occurrence among the patients involved in this study. The mean admission duration length was 6 days. The highest number of MRSA occurred in the patients who stayed (4-6) days in the hospital and its burden was (36,63.2%) and the least was seen among patients who stayed (13-15) days in the burden of MRSA was(0,0.0%)(Table-8) (P value=0.43).

Table-8: Occurrence of Staphylococcus aureus (MSSA) and MRSA isolates in admitted patients in relation to duration of hospital admission length in days between January 2018 up to January 2019 (n=160)

Duration groups in days	**MSSA**	**MRSA**	**CoNS**	**Oxacillin Resistance CoNS**	**No growth**	**Total**	**P value**
1-3	7(1.7%)	7(1.7%)	11(2.7%)	3(0.7%)	29(7%)	57(13.8%)	
4-6	66(16%)	36(8.7%)	25(6.1%)	7(1.7%)	105(25.4%)	239(57.9%)	**P<0.43**
7-9	24(5.8%)	12(2.9%)	9(2.2%)	2(0.5%)	40(9.7%)	87(21.1%)	
10-12	4(1%)	2(0.5%)	1(0.2%)	0(0.0%)	15(3.6%)	22(5.3%)	
13-15	2(0.5%)	0(0.0%)	1(0.2%)	0(0.0%)	5(1.2%)	8(1.9%)	
Total count	103	57	47	12	194	413	
Total %	24.9%	13.8%	11.4%	2.9%	47%	100%	

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7. Discussion

S. aureus is known as an important bacterial pathogen that can cause community and hospital-acquired infections with high morbidity and mortality rate in spite of the use of antibiotics. In the current study, *in vitro* susceptibility pattern of this gram-positive pathogen was assessed using clinical specimens from patients suspected of hospital acquired infections in (Hematology, Orthopedics, surgery, ICU, gynecology and pediatrics) who showed sign and symptoms of nosocomial infection after 48 hours of admission were included in the study randomly.

In the present study, among the isolates of MRSA, males account (35, 61.4%) had a higher isolation rate than (22, 38.6%) females. The prevalence of MRSA in the present study, however

did not vary significantly by gender where (P=0.4) (Table-5) and although there was a slight variation of MRSA recovery in different age groups but age was not associated with the acquisition MRSA where (P=0.8) (Table-4).

In this study, the prevalence of *Staphylococcus aureus* isolation rate was found to be (160/413, 38.7%) among the study population which was in agreement with a similar study conducted in Debre Markos Hospital (34) its prevalence was (39.7%), and higher than the study conducted in Mekele (81, 32.5%) (30) and less than the study conducted in Pakistan(110, 55%) (28) and Yekatit hospital(57.8%), (160 ,82.5%)respectively (62 and 35). Out of the 160 S. aureus isolates in our study (57, 35.6%) were MRSA but the total burden among the study population were 13.8%. This variation in prevalence in different countries and health institutions may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to Hospital (63).

The highest level of antimicrobial resistance among *S. aureus* isolates in this study was observed in penicillin 143(89.4%), gentamyicin 87(54.4%), ciprofloxacin 89(55.6%), erythromycin 93(58.1%), Trimptoprim- sulfametaxazole 69(43.1%), clindamyicin 9(5.6%), Augumentinand other Beta Lactamase including Carbapenums were 57(35.6%)and it was in concordance with the reports of (28 and 37). The resistance MRSA to ceftriaxone in this study was57(100%) inconsistent with the study reported in Mekele which was (75%) (30).

In our study, we found high percentage of erythromycin-resistant *S. aureus* isolates among MRSA patients 56(98.2%). Among them 7(12.3%) isolates tested positive for clindamycin induced erythromycin resistance by D-test, while rest of the isolates were negative for D-test. The findings are contrast with the previous studies conducted in India and these observations suggest that if D-test not been performed, one-eighth of the erythromycin-resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure (41).

In our finding, Clindamycin resistance among MRSA isolates were (16, 28.1%). The prevalence of MLS_B-inducible resistance among MRSA clones is not widely reported. In a **Brazilian** study, 11.3% of *S. aureus* isolates were shown to have this phenotype, whereas (7, 12.3%) of MRSA isolates tested positive for MLS_B-inducible resistance in a Turkish study (68&69). In Our findingMLS_B-inducible resistance among MRSA isolateswas(9, 15.8%). It was in concordance

with the finding of at Yekatit hospital (23, 11.9%) (35) and Brazilian study (7, 12.3%) (58).The increasing prevalence of methicillin resistance among *Staphylococci* is an increasing problem. This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogram B (MLS_B) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties (56,57 &58). However, widespread use of MLS_B antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLS_B antibiotics (58, 59 & 60).

Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible (*60*). The most common mechanism for such resistance is target site modification mediated by *erm* genes, which can be expressed either constitutively (constitutive MLS_Bphenotype) or inducibly (inducible MLS_Bphenotype). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other. In such cases, *in vivo* therapy with clindamycin may select constitutive erm mutants leading to clinical therapeutic failure. In case of another mechanism of resistance mediated through msrA genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both *in vivo* and *in vitro* and the strain do not typically become clindamycin resistant during therapy. (58).

In the present study we conducted, Regarding the resistance profile of isolates to individual drugs indicated that *S. aureus* showed an average resistance rate of 87.6% to most of the antimicrobial drugs tested (Table -6) which showed relatively higher resistance than the previous studies done elsewhere in Ethiopia where average resistance of>65% were recorded (30 *and 43).*
Many factors may have contributed to the above level of resistance towards the tested antibacterial drugs, including misuse of antibiotics by health professionals, drug terminations by patients, contaminated blood transfusion, contact with pets and lack of sanitations etc.
In Ethiopia it is a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public, thus contributing to the emergence and spread of antimicrobial resistance. Other causal factors could be poor hospital hygienic conditions, accounting for the spread of resistant bacteria and inadequate surveillance, i.e. lack of information from routine antimicrobial susceptibility testing of bacterial isolates and surveillance

testing of bacterial isolates and surveillance of antibiotic resistance, all of which are crucial for good clinical practice and for rational policies against antibiotic resistance (47).

In our study the overall burden of MRSA infection was (13.8%, 57/413). It was by far less than the study reported in TikurAnbessaHospital (28.7%) (43), Debre Markos hospital (19.9%) (34), Bangladesh (33.9%) (27), (39%) in Moi teaching and referral hospital kenya (29), (26%) in Venezuela, Latin America (42) and incomparably higher than finding in Mekele(2.4%) (30), and Yekatit Hospital (34, 2.5%) (35).

This finding was also higher than other studies conducted in Italy 1.2%, in Germany 2.5% and in Madagascar 1.3% (32,33 &44) respectively. This variation may due to strong hospital acquired infection prevention and drug monitoring policy.

In the current study we did, the highest rate of MRSA was observed among Hematology ward patients 16/57(28. %) followed by Surgery 15/57(26%), ICU 10/57(17.5%), Pediatrics 8/57 (14%), Gynecology 4/57 (7%), and the least was observed in Orthopedics and its prevalence was 4/57(7%)(table-7). Acquisition of MRSA was significant with patients admitted in different wards (where P value <0.05.) As compared with (53) and (49) the prevalence of the current MRSA in the source patients were almost less than by half.

The highest prevalence among inpatients were expected due to the long hospital stay, ward conditions such as changing of clothes, sneezing, nose picking and other personal habits like poor hygiene, which pollute every patient in the wards, transfusion of unscreened blood and blood products, occurrence of bed sore, medical indwelling devices and previous admission history. In this study setting, acquisition of MRSA hasn't affected by long term duration of hospital stay and it was not statistically significant (where P Value<0.05). Transmission of MRSA occurs primarily from colonized or infected patients or health care workers to other patients or vice versa. Among the resistant pathogens, MRSA was the greatest concern because of its particular importance in causing various clinical conditions. Therefore, the risk of acquiring *S. aureus* infection were increased in the wards in the presence of other hospitalized shedders 'who may be possibly infected with the antibiotic resistant strains (51).

In Our study, the overall MDR rate of *S. aureus* isolates among MRSA were 83.6% (resistant to three to nine drugs). This finding was not in concordance with 79.6% and 65.2% reported in Gondar respectively (43&48). In contrast, our finding was less than 98.6% and 100% MDR reported in Ethiopia respectively (50&52).

Most common reason for multi-drug resistant *MRSA* is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients' poor economic status, poor hospital hygiene, personal hygiene, contact with pets and unscreened blood and blood products transfusion. In this study, high prevalence of multidrug resistant MRSA predispose patients to infection with intractable isolates and emphasizing the need for improved infection control practices and guidelines for use of antibiotics in this setting. Moreover, all MRSA strains isolated in this investigation were resistant to ≥5 antibiotics tested which was in agreement with (52) in which almost 100% of MRSA strains were multidrug resistant. This indicated that resistant strains were emerged and the emergence of those resistant strains, especially for the most bactericidal anti-MRSA agents, may have further aggravated the emergence of multidrug resistant MRSA, and it may threaten the success of a MRSA control program. In this finding acquisition of MRSA was related with previous history of drugs taken with in the last six months, hence it was statistically significant (Value<0.05) (Table-2).

The current study was by far less than the study conducted in Uganda showed that from the isolates studied 8 (25%) were MRSA but in our study, 57(13.8%) from a total of 413 study participants were MRSA. In this study, (52, 91.2%) of the isolates were resistance to Gentamicin which was in agreement with the study conducted in Uganda that showed (87.5%) were sensitive to gentamicin **(**7**).**

Another study conducted in the same country, in Lacor hospital, revealed that *S. aureus were resistance to* Ampicillin (81.5%), Erythromycin (10.5%), Gentamycin (10%), Methicillin (2.6%) (51) but our finding in general by far not in concordance i.e Penicillin, Erythromycin, Gentamycin, Methicillin were (89.4%, 58.1%, 54.4% and 35.6% resistance respectively). Our study was also higher than studies conducted Kenya, 71% *S. aureus* isolates have demonstrated multiple drug resistance **(**65)andin contrastStudy done in Gondar university, Ethiopia revealed that staphylococcus bacteria showed low level of resistance (<60%) to all antimicrobials tested which was similar with our finding AMC=Amoxicillin-clavulanic acid; CRO=Ceftriaxone; GN= Gentamicin; E=Erythromycin; P= Penicillin and SXT= Trimethoprim- sulphamethoxazole (64)**.**

Control of MRSA in healthcare settings relies on understanding factors that predispose patients to the acquisition. Risk factors for HA-MRSA acquisition have been well described and include, invasive procedures, long hospital stay, antibiotic exposure and use of medical devices. The

strongest risk factor for HA-MRSA infection in our setting were drugs taken with in the last six months and admission in different medical wards (where p value<0.05) (Table 1 &2). To the best of our knowledge, this is the first multi-center study that included patients of all ages admitted in a variety of wards to demonstrate this independent association.

A similar Previous studies done in two tertiary hospitals in South African provinces found that *S. aureus* was the major pathogen infecting patients in burn units, and *MRSA* accounted for 66% of these infections in KwaZulu-Natal Province and 58% in Eastern Cape Province (54). A similar study of patients with bloodstream infections and severe burns conducted in Gauteng found that 35% of these infections were due to *MRSA*(37).

The World Health Organization (WHO) has reported that there is a higher burden of hospital-associated infections in low- and middle income countries, and as opposed to adults in high-income countries, neonates are most at risk in low- and middle-income countries, with up to 20 times higher infection rates (41).Thus, our findings support those of WHO emphasis, the need to control MRSA acquisition in neonates and patients that have breach in their body, as they contribute the most to the burden of disease and are likely to have unfavorable outcomes.

8. Conclusion and Recommendation

It is known that antimicrobial resistance is a growing global problem. In conclusion the increased proportion of *MRSA* isolated in this study was considered as an alarming because only a few treatment options remain for S. a*ureus* infections. About 13.8%(57/413) of *S. aureus* was oxacillin/cefoxitin resistant (*MRSA*). Although Clindamycin showed a low rate of resistance our study, Vancomycin, Ceftaroline, linezolid and Daptomycin are choices of antimicrobial agent available to treat life-threatening infections with *MRSA*.

Multiple drug resistance of *Staphylococcus aureus* isolates to antimicrobials was alarmingly high so that any empirical prophylaxis and treatment needs careful selection of effective drugs. Also there should be continuous monitoring of the antimicrobial susceptibility pattern of methicillin resistance *staphylococcus aureus* for the selection of appropriate therapy, developing the antibiotic policy and limiting the use of powerful antibiotics is vital.

The prevalence of *MRSA* is relatively higher in TikurAnbessaSpecialised hospital, in Ethiopia. Several identified risk factors of MRSA infections should be considered when instituting infection and prevention strategies in public-sector hospitals, including intensifying the implementation of antimicrobial stewardship programs.

There is an urgent need to strengthen infection prevention and control in Hematology, surgery, Gynecology, orthopedics, neonatal wards, and intensive care unit's patients. To minimize such resistant infections, adherence of strict aseptic surgical procedures and proper management of wound is required.

Further detection and molecular characterization of the gene (mec A), phage typing and analyses of the plasmids of MRSA is necessary. Strict consideration for *staphylococcus aureus* infection and proper usage of antibiotic policy are recommended in decreasing the incidence and occurrence of multidrug resistant *staphylococcus aureus* infections in TikurAnbessaSpecialised Hospital. It is also necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in this hospital to prevent the spread of resistant MRSA.

In conclusion, each patient should be screened before admission for MRSA carriage to prevent the spread of the resistance gene in the facility.

9. Limitation

One of the limitations of this study was MIC testing for cefoxitin resistance coagulase negative staphylococcus was not done because of unavailability of the kits during the study period.

E-test for vancomycin resistance among the isolates of *MRSA* was not performed to know the susceptibility patterns of the isolates.

There were no similar studies done in Ethiopia to know the patterns of *MRSA* distribution in a wider scope among admitted patients in different wards, who were suspected for hospital acquired infection to know the burden.

However, this study is a pragmatic one, given that the study area in particular in Ethiopia, antibiotics are prescribed on empirical bases without implementing the commonly recommended strain isolation and susceptibility testing procedures.

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10. Annexes

Annex I- English version of participant information sheet, consent form and data collection sheet

2.1. Participant information sheet**:** Name of the organizationDepartment of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

My name is----------. I am working as data collector for the study being conducted in this Hospital byTewodrosTamire who is studying for his Master's Degree in Public health and Diagnostic Microbiology at Addis Ababa University Department of Medical Laboratory Science, School of Allied Health Sciences, Collage of Health Sciences. I kindly request you to lend me your attention to explain you about the study and being selected as the study participant.

Title of the Research Project: The prevalence of methicillin resistance staphylococcus aureus among admitted patients in (ICU, Hematology, Surgery, Gynecology, Pediatrics and Orthopedics) units in TikurAnbessa Specialized Hospital**.**

Name of Investigator: TewodrosTamireAbiche**.** First of all, we would like to thank you in advance for your cooperation and consent in participation in this study. Please take as much time as you need to read or listen the information sheet. If you have any question regarding the study, please ask freely.

Background information

Staphylococcus aureus is becoming multi drug resistance infectious health problems in the world including in developed and developing countries which are the most important aggravating agents of mortality, morbidity, length of hospital stays and cost in the world. It is a frequent health problem particularly in intensive care units (ICU), surgery and in other units because of various therapeutic or diagnostic interventions those are frequently and for extended period used such as the use of wide spectrum antibiotics, mechanical ventilation, central venous catheterization, invasive pressure monitoring and urinary catheterization. The burden of health care associated infection in developing countries is high prevalence of HCA infection is much higher than proportions reported from developed countries. In developing countries, the risk is

two to twenty times higher and the proportion of infected patients frequently exceeds 25%. In low and middle income countries, the burden of hospital acquired infection is unknown due to lack of reliable data. Staphylococcal infection is expected high but given less attention has been given in Ethiopia. This problem is one of the health's rated problems in Ethiopia. Relatively, few data are available from Ethiopia. Moreover, several bacterial nosocomial infection carries a substantial economic burden due to high antimicrobial use and increased length of hospitalization. This burden of resistance, however, is probably more due to the high rate Antimicrobial drug resistance is a major global problem affecting both developed and underdeveloped countries as well as problem of both in the community and health institutions. Along with the problem of nosocomial infection comes with the burden of "multidrug" antimicrobial resistance. The ongoing emergency of resistance in the community and hospital is considerable a major threat for public health.

Purpose of the Research Project

We are asking you to take part in this study because we are trying to learn more about multi drug resistance staphylococcal infections and the drug susceptible profile in children and adults suffering from staphylococcal infections .More over The finding of this study study will have para amount importance in prevention and control of staphylococcal hospital acquired infections and in planning hospital antibiotic policy at TikurAnbesa Specialized Hospital, Collage of health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Potential benefits to subjects and/or to the society

You will not have any financial incentives or other inducements as the compensation from participating in the study. However, results will be given to their physician for treatment and management or to get counseling. Most importantly, the result of the study will be beneficial to provide information or data for future and further nationwide study and to develop health programs for health policy makers. Hence, you are indirectly benefiting other patients and the society in this respect.

Risks and complications

You will not be at any physical or psychological risk but during collection of the sample you may feel small discomfort, this does not produce serious pain.

Confidentiality

In order to keep the confidentiality of the participants the sample will be labeled with code instead of giving name. No personal information will be disclosed to third party or will not appear in any report from this study. You can choose whether to be a part of this study or not and you have a right to get a laboratory diagnosis result for free

Rights:
Participation for this study is fully voluntary. You have full right to either participate or not in this study and it will never affect your right of getting appropriate treatment. If you feel uncomfortable with data and sample collection process, you have full right to withdraw the data and sample collection process at any time.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project. If you have any question you can contact and ask at any time you want. You are always well come at the following address given bellow.

Principal investigator

NAME: TEWODROS TAMIRE

Address Department of Medical Laboratory Sciences, Collage of health Sciences, Addis Ababa University, and Addis Ababa, Ethiopia

Mobile +251911977594 E-mail: tewodrostamire94@gmail.com

2.2 Informed consent

I have read /was read tome that the participant information sheet. I have clearly understood the purpose of the research, the procedure, the risks and benefits, issues of confidentiality, the right of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I was informed that I have the right to with draw from the study at any time or not answer any question that I do not want. Therefore, I declare my voluntary consent to participate in this study with my signature as indicated below.

Name of participant ----------

Signature of participant: ----------Date-------/-------/----------

Signature of data collector: ----------Date------/-------/---------

2.3 Laboratory data collection form

1. Code no_____

2. Age _____Sex _____ Date_____

3. Types of specimen: blood or pus /both

4. Media used

_____

5. Organism isolated

_____

Culture and biochemical tests identification

_____

_____

7. Gram stain from specimen_____

8. Result of Gram stain from culture_____

9. Antimicrobial test

Sensitive to _____

Intermediate to_____

Resistance to_____

Comments

Name of principal investigator _____ Signature

_____ Date_____

Annex II- Information Sheet and Informed Consent Form

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

You are invited to participate in a study to be conducted by MSc student at Addis Ababa University, College of Health Sciences and Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction**:** The topic of this study is determining multi drug resistance staphylococcal infections in admitted patients in a tertiary care teaching hospital in Addis Ababa, Ethiopia. Participation in this study is exclusively voluntarily. If you are not interested to participate, there will be no consequences.

What is expected from me as participant of the study?

As a participant of this study, there is no additional clinical sample (blood, urine, swabs, sputum etc.) taking from you. The left over sample will be used for this study.

Potential benefits to participant and/ or to the society

Based on the results obtained from the study, corrections will be taken in diagnosing multi drug resistance staphylococcal infections. Hence, you are indirectly benefiting other patients and the society at large.

Compensation for participation

You will not receive any payment for your participation in this research study.

Confidentiality

On the request paper your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose.

Person to contact

Please direct any questions you may encounter during this study to the principal investigator.

Principal investigator

TEWODROS TAMIRE

Address Department of Medical Laboratory Sciences, Collage of health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Mobile +251911977594 E- mail: tewodrostamire94@gmail.com

Consent form

This page contains an agreement signature to participate in the study entitled **"**The prevalence of MRSA and associated factors among admitted patients in black lion tertiary care hospital Addis Ababa Ethiopia**." S**o please read the following points and sign your signature at the end in the space provided.

I understand the objective of the study inthe prevalence of MRSA and associated factors among admitted patients.

1. I know that the left over sample (blood. urine, swabs, pus etc) that I will give is going to be used for this study only.
2. I understand that, all the information and the results are confidential.
3. I understand that I will not get any money for my participation.
4. All the information is explained by reception, phlebotomist and Principal investigator.

Therefore, with full understanding of the situations I agree to give a sample for laboratory analysis.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Accent form

This page contains an agreement signature to participate in the study entitled **"**The prevalence of methicillin resistance staphylococcus aureus among admitted patients in Black Lion tertiary care Hospital Addis Ababa Ethiopia." So please read the following points and sign your signature at the end in the space provided.

I understand the objective of the study inthe prevalence of methicillin resistance staphylococcus aureus amongadmitted patients at TikurAnbesa Specialized Hospital, Addis Ababa, Ethiopia.

1 I know that the left over sample (blood, urine, swabs and pus etc.) that my child gave is going to be used for this study only.

2. I understand that, all the information and the results are confidential.

3. I understand that my child will not get any money for my participation.

4. All the information is explained by reception phlebotomist and Principal investigator.

Therefore, with full understanding of the situations I agree for my child to give a sample for laboratory analysis.

Signature of the participant's parent: _____

Address of the participant: _____

Date: _____

Annex-III. Questionnaire (English version)

The purpose of this Questionnaire to study The Prevalence of Methicilin Resistance Staphylococcus infections andassociated risk factors among admitted patients suspected forHAI at TikurAnbesa Specialized Hospital, Addis Ababa, Ethiopia. Your responses will be used for the purposes of research for partially fulfillment of the Requirements for the Degree of Masters. So you are kindly requested to give your response.

Data collection form for assessing the prevalence of multidrug resistance staphylococcal infections among admitted patients.

Instruction: Read the questions audible and give your appropriate response

I. Patient information		
SN	**Questions**	**Coding categories**
1	Ward	_____
2	Age(in years)	_____
3	Sex	1.Male 2.Female
4	Date of admission in the hospital	dd /mm/yy
5	What is your marital status?	**1** Single 2. married 3. Widowed 4. divorced

II. ward's Facility	
1. Do you think the ward is clean?	1.yes 2. No
2. Do you think the ward is ventilated?	1.yes 2. No
3.Do you think the health workers personal protective equipment is clean?	1.yes 2. No
4. Do you think staffs wash their hands before after contact with the patient?	1.yes 2. No

5. Do you think health workers use sterilized medical equipment?	1.yes 2. No

III. Patient pre-admission History

1. Do you have prier history of admission? 1. Yes 2. No

2. If your answer for question no. 1 is yes what was the reason for admission?

1. Surgery 2. Medical 3. Delivery 4. Emergency

3. What was hospital's sanitation condition? 1. Excellent 2. Very Good

3. Good 4. Poor

4. Were the medical equipment Sterilized? 1. Yes 2. No

5. Did you saw that health professionals used gloves to treat you? 1. Yes 2. No

6. Did you used any medical equipment's in your body such as like Catheter, Drug lines. etc.?

1. Yes 2. No

IV. **PATIENT POST MEDICAL SERVICES HISTORY**

1. Do you have wound infections? 1. Yes 2. No

2. If your answer is yes, what is the cause of the wound?

1. Surgical 2. Burns 3. Bites (insect, animal or snake) 4. Accident 5. Others (specify)…

3. Do you have a history of blood transfusion? 1. Yes 2. No

4. Have you been admitted nursing home or transferred from another hospital? 1. Yes 2. No

5. Have you been in hospital with in the last six months? 1.Yes 2. No

6. Did you take medicine without your physician order? 1.Yes 2. No

7. Did you terminate taking medicines without your physicians order 1. Yes 2. No

8. Did you take medicines below or above length of dates prescribed? 1.Yes 2. No

9. Do you have any chronic lesions? (e.g. leg ulcers, wound) 1. Yes 2. No

10. How long days did you stay in the hospital? 1.Yes 2. No

11. Did you have a new infection within the last 48 hours after admission? 1.Yes 2. No

12. If your answer for question no.11 is yes, what was the cause?

A. surgery B. Old infection C. unknown

13. Do you feel pain in your urinary tract? 1.Yes 2. No

14. Do you feel chest pain? 1. Yes 2. No

15. Do you have respiratory tract infection? 1. Yes 2. No

16. Do you have recent or past bedsore infection? 1. Yes 2. No

17. Do you have an indwelling device? (like urinary catheter). 1. Yes 2. No

18. Do you have a current or past history of IV lines usage? 1.Yes 2. No

19. Are you an intravenous drug user? 1. Yes 2. No

20. Do you live currently or in the past in a health care staff hall? 1. Yes 2. No

21. Did you have contacts with pets? 1. Yes 2. No

22. Do you a history of any organ transplant? 1. Yes 2. No

23. Do you have a history of surgery around your sphincter? 1. Yes 2. No

24. Do you have a history antibiotics prescribed in the last 6 months? 1.Yes 2. No

25. If your answer for question no. 24 is **yes,** mark **x** in the box for the drug/drugs you used.

Antibiotic s	Mark x
Amoxicillin	
Augmentin	
Erythromycin	
Ceftriaxone	
Clindamycin	
Ciprofloxacin	
Azithromycin	
Cloxacillin	
Vancomycin	

If others specify_____

THANKS A LOT FOR YOUR PARTICIPATION!!!

Gram stain

i. This will be used to differentiate Gram positive (appears purple) and Gram negative

(appears pink) bacteria. The following steps will be followed.

ii. Fixing the dried smear by passing over a flame three times.

iii. The fixed smear will be covered with crystal violet for 30-60 seconds.

iv. The stain will be rapidly washed with clean water.

v. All the water will be tipped off and the smear covered with grams' iodine.

vi. The iodine will be washed with clean water.

vii. The smear will be decolorized rapidly (in a few seconds) with acetone alcohol, then

washed with clean water.

viii. The smear will be covered with neutral red stain for two minutes.

ix. The stain will then be washed off with clean water.

x. The back of the slide will be wiped clear and placed in a draining rack for the smear to air dry.

xi. The smear will then be examined microscopically first with 40x objective to check the staining and see the distribution of materials and then in oil immersion objective to look for bacteria and cells.

Catalase test

It Will be used to differentiate the bacteria that produce the enzyme catalase such as *staphylococci* from non-catalase producing bacteria such as *streptococci.*

Method

i2-3ml 0f hydrogen peroxide solution will be poured into a test tube.

ii Using a wooden stick or a glass rod several colonies of the test organism will beremoved and immersed in the hydrogen peroxide solution.

iii. Active bubbling indicates a positive catalase test.

Coagulase test

This test will be used to identify staphylococcus aureus which produces coagulase. Both tube test and slide test will be employed.

Method

Slide test (detects bound coagulase)

i. A drop of distilled water will be placed on each end of a slide or on two separate slides.

ii. A colony of the test organism will be emulsified in each of the drops to make two thick suspensions.

iii. A loop full (not more than) will be added to one of the suspensions and mixed gently.

iv. Clumping of the organisms will occur within 10 seconds if the organism is *Staphylococcus aureus.*

v. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Tube test (detects free coagulase)

i Plasma will be diluted in the ratio of 1:10.

ii. Three small test tubes will be availed and labeled; test organism, positive control and negative control.

iii. 0.5ml of the diluted plasma will be pipetted into each tube.

iv. Five drops (about 0.1ml) of the test organism will be added into the labeled positive and 5 drops of the *Staphylococcus aureus* culture will be added to the tube labeled positive and 5 drops of sterile broth in the tube labeled negative.

v. The tubes will be incubated at 35-37C after mixing gently. Clotting will occur after an hour, if no clotting occurs after an hour examination will be repeated after every 30 minutes for up to 6 hours.

vi. Clotting is indicative of Coagulase + ve*Staphylococcus aureus.*

Antimicrobial sensitivity testing- Disc diffusion method.

A disc of blotting paper is impregnated with known volume and appropriate concentration of an antimicrobial. The disc 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 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 the edge of the disc. To ensure reproducibility and comparability of results, the modified Kirby-Bauer diffusion technique will be used.

Modified Kirby-Bauer susceptibility testing technique

A sterile medium will be prepared according to the manufacturer's instructions. The PH of the medium will be set at 7.2-7.4. The media will be poured into a 90mm sterile petri dish to a depth of 4mm (about 25ml per plate). This will be done on a level surface so that the depth of the medium is uniform. NB If the media is too thin the inhibition zone will be falsely large and if too thick the zones will be falsely small. Each new batch of agar will be controlled using *E. faecalis*(ATCC 29212 or 33186) and cotrimoxazole disc. The zone of inhibition should be 20mm or more in diameter. The plates will be stored at 2-8^{o}C in sealed plastic bags. For use the plates will be dried with their lids slightly raised in 35-37^{o}C incubator for about 30minutes. About one hour before use, the working stock of the discs will be allowed to warm to room temperature, protected from direct sunlight.

Method

1) Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance to the test organism and emulsify in 3-4ml of sterile physiological saline or nutrient broth.

2) In a good light match the turbidity of the suspension to the turbidity of the 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 rotating and pressing 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^{o}C to ensure even distribution.

4) With the petri dish lid in place, allow 3-5 minutes (no longer than 15minutes) for the surface of the agar to dry.

5) Using sterile forceps, needle mounted in a holder, or multidisc dispenser, place appropriate antimicrobial discs, evenly distributed on the inoculated plate. The discs should be 15mm from the edge of the plate and no closer than about 25mm from disc to disc. No more than eight discs will be applied on each petri dish. Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved in one place.

6) Within 30minutes of applying the discs, invert the plate and incubate it aerobically at 35^{o}C for 16-18 hours.

7) After overnight incubation, examine the control and the 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 of zone sizes

Using the interpretative chart, the zones of each antimicrobial will be interpreted reporting each organism as Resistant, Intermediate susceptible, Susceptible.

Antibiotic Sensitivity of Staph. aureus

Antibiotic	Resistant	Intermediate	Sensitive
Clindamycin			
Penicillin			
Augumentin			
Erythromycin			
Ceftriaxone			
Ampicillin			
Ciprofloxacin			
Cefoxitin			
Azithromycin			
Gentamycin			

Sensitive zone of radius is wider or equal to the control.

Intermediate zone of radius is more than three mm smaller than the control.

Resistance no zone of inhibition

Isolated organism	AST	Drugs	AMX	AMK	STX	FOX	CTX	GE	ERY	OX	CRO	AMC	CLI

	S												
	I												
	R												

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF METICILLIN RESISTANCE STAPYLOCOCCUS AUREUS AND ASSOCIATED FACTORS AMONG PATIENTS SUSPECTED FOR HIA IN ADMITTED PATIENTS IN TIKUR ANBESSA SPECIALIZED HOSPITAL.

Principal Investigator

TEWODROS TAMIRE

Signature _____ Date_____

Advisor:

KASSU DESTA (MSC, PhD Fellow, Associate Prof.)

Signature _____ Date_____

May, 2019

Addis Ababa, Ethiopia

Declaration

The undersigned declares that this thesis dissertation paper complies with the regulations of the University and meets the accepted standards with respect to originality and quality. The PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: TewodrosTamire (B.Sc.)

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisor.

Advisor:

KassuDesta (MSc, PhD Fellow, Associate Prof.)

Signature: _____

Date: _____