NASAL CARRIAGE RATE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG CLINICALYEAR MEDICAL STUDENTS OF JIMMA UNIVERSITY, SOUTH WEST ETHIOPIA

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Abstract

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) nasal carriage is a potential niche for spread and a risk factor for subsequent infections. However, there is limited data on nasal carriage rate of *S. aureus* and MRSA among clinical year medical students in Africa and none in Ethiopia.

Objective: To determine the prevalence of nasal carriage rate of methicillin resistant *Staphylococcus aureus* and contributing factors for colonization of MRSA among clinical year medical students of Jimma University, southwest Ethiopia.

Method: A cross-sectional study was conducted among 371 clinical year medical students, (clinical-I, n=166 clinical-II, n=125 and medical-intern n=80) who had been on clinical practices at Jimma University Specialized Hospital (JUSH) from May to August, 2016. Nasal swab was taken from all eligible subjects with sterile cotton swabs. Samples were processed for identification of *S. aureus*and MRSA.Antimicrobial susceptibility was done according to standard operating procedures and data was analyzed using SPSS version 20.

Results: A total of 82 *S. aureus*isolates were recovered from 371 samples. The overall prevalence of*S. aureus*and MRSA among the study population was 22.1% and 8.4% respectively. Length of hospital practice and MRSA colonization showed statistically significant association.Penicillin and ampicillin showed 100% resistance to MSSA isolates while clindamycin sensitivity was high to all isolates. In addition 52.9% MSSA and 48.4% of MRSA isolates wereshowed multidrug resistance.

Conclusion: This study shows that the carriage rates of *S. aureus* and MRSA among medical interns was high. We conclude that clinical exposure may increase colonization by MRSA.According to this study clindamycin is effective treatment against MSSAand MRSA.Alcohol-based hand rub antiseptics should be placed strategically in the hospitals.

Key words: MRSA, clinical year medical students, nasal carriage, *S aureus,*Ethiopia

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Abbreviations/Acronyms

ATCC: American Type Culture Collection

CA-MRSA : Community associated methicillin resistant staphylococcus aureus

CDC : Centers for Disease Control

CLSI : Clinical Laboratory Standards International

HAI: Hospital Associated Infection

HA-MRSA: Hospital acquired methicillin resistant staphylococcus aureus

HCW: Health care worker

JUSH : Jimma University specialized Hospital

MDR: Multidrug Resistant

MIC: Minimum Inhibition Concentration

MRSA: Methicillin resistant staphylococcus aureus

MSSA: Methicillin Sensitive Staphylococcus Aureus

PBP2: Penicillin-binding protein Two

SCCmec: *Staphylococcus* Cassette Chromosome mec

WHO: World Health Organization

Operational definition of terms

Clinical year medical students; medical students who are attending 4^{th},$5^{th}$and 6^{th} year medicine.

Clinical –I; 4th year medical students

Clinical –II; 5^{th} yearmedical students

Medical intern; 6^{th} year medical students

High decontamination score =always using sanitizers

Moderate=using sanitizers sometimesor always water and soap

Low =always using water only or water and soap sometimes

CHAPTER ONE: INTRODUCTION

1.1. Background

Staphylococcus aureus is non-motile, non-spore forming facultative anaerobic gram positive cocci arranged in cluster (1). It is part of the most frequent normal flora of human skin and nasal passages (2). Although multiple body sites can be colonized, the moist and lower temperature environment of the squamous epithelium of the anterior nares appears to be the main ecological niche(3).

Staphylococcus aureus cause a range of illnesses from minor skin infections and abscesses, to life-threatening diseases such as, meningitis, endocarditis, toxic shock syndrome, and septicemiaNasal colonization is responsible for the fast spread of the staphylococcal infections and this situation seems worse in hospital setup causing hospital-associated infections (HAI) such as; surgical site blood stream infection. The bacterium can also invade any tissue in the body, causing other serious life-threatening diseases such as osteomyelitis, and pneumonia in hospitalized patients(4,5).

Methicillin resistance *S. aureus* (MRSA) is those strains of *S. aureus* that express mecA gene and encode the penicillin-binding protein 2a (PBP 2a) (6). Those isolates that are positive either for mecA or PBP 2a classified as methicillin resistance or reported as oxacillin resistance, cefoxitin resistance or modified *S. aureus* strains (7). MRSAevolves mainly due to the accumulation of point mutations and selection, and to a minor degree due to the horizontal gene transfer of mobile genetic elements originating from the same (intra-species) or different species (inter-species)(8,9)

MRSA strains are resistant to virtually all β-lactams with the exception of the latest generation of cephalosporin β-lactams. MRSA can also resistance to other multiple classes of antimicrobials. Moreover, *S. aureus* is the pathogen of the greatest concern because of its inherent virulence, easily circulated in the environment, its ability to acquire genes encodes for biofilm formation and its capacity to adapt different environmental conditions (10,11)

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There are two kinds of MRSA have been described: Hospital- associated MRSA (HA-MRSA) and Community-associated MRSA (CA-MRSA). The infections caused by HA-MRSA and CA-MRSA are generally different; The SCCmec types I, SCCmec type IV and SCCmec V, which associated with CA-MRSA usually carry no additional drug resistance genes other than mecA. That is why the CA pathogen is most frequently associated with skin and soft tissue (abscesses, boils, and folliculitis) andmore frequently susceptible to non β-lactam antibiotics than HA-MRSA(8). SCCmec types II and III isolates carry additional genes that provide resistance to heavy metals and drugs other than β-lactams which makes HA-MRSA more resistant to other antimicrobial agents in addition to β-lactams(12). And HA pathogen is more likely to infect the respiratory tract (pneumonia), blood stream bacteremia (septic shock), cellulitis, endocarditis, and urinary tract(13).

Asymptomatic *S.aureus* nasal carriage is a major risk factor for multiple types of suppurative endogenous infections as well as bacterial transmission both in private and nosocomial environments (14). Nasal carriage of *S. aureus* among health personnel is an important source of Hospital Associated Infection (15). Health care workers, who have direct contact with persistently colonized patients, or contaminated objects in the immediate environment around them can contaminate their hands and subsequently transmit the organism to other patients (16). A subset of these will remain as nasal carrier for a prolonged period of time may spread the organism to patients by direct contact transmission (17,18).

Carriage rate varies widely from place to place in the community and hospital setting including health care workers(19). The majority of MRSA colonization and infections occur in hospitals and other health care settings (20). *S.aureus* nasal carriage state among medical students at present shows MRSA isolates are the most frequent cause of complicated nosocomial infections(13,21). reports shows that there is high burden of MRSA among Ethiopian hospitalized patient(22,23) Thus, identification of asymptomatic MRSA-carriers is one of the most important measures to reduce the risk of nosocomial transmission of MRSA-infections and information regarding the prevalence of nasal carriage of MRSA and factors associated with such carriage could be an important step for prevention of MRSA spread and nosocomial infections.

1.2. Statement of the problem

The anterior nares are the primary colonization site of *S. aureus* and *S. aureus* carriage is a known risk factor for *S. aureus* infection(24). *Staphylococcus aureus* is one of the commonest human pathogens capable of causing a wide range of infections Worldwide and it is the most causes of nosocomial infections with high morbidity and mortality rates(24). Globally, an estimated 2 billion people carry *S aureus* of these, up to 53 million (2.7%) are thought to carry MRSA(28). In the United States only,95 million carry *S. aureus* in their noses; of which 2.5 million (2.6%) carry MRSA and Out of 80,461 invasive MRSA infections, 11,285 related deaths occurred in 2011(29).

S aureus ranks the second cause of nosocomial bloodstream infections, which leads to increased morbidity mortality, hospital stay and costs in United States. Recent data shows that 82% of nosocomial bacteremic *S. aureus* strains are endogenous and originate from the nose of the carriers(30).

According to Centers for Disease Control (CDC) and World Health Organization (WHO) 2015 reports, MRSA incidence was declined in Europe, the United States and Canadian over the past eight years(25) Whereas in sub-Saharan Africa, India, Latin America, and Australia, it is still rising (24, ,25, 26). MRSA resistance rates exceed 20 percent in all WHO regions and are above 80 percent in some regions like sub-Saharan Africa (26).In Ethiopia, reports from different hospitalsand pooled prevalence of MRSA showed that the overall estimation prevalence of MRSA in Ethiopia is from 24.1% to 40.9%(21,29)

Nasal carriage MRSA is widespread in patients, hospital staffs and health students who had been in clinical practices and carriers are more susceptible to develop skin sepsis postoperative infection, Pneumonia and also may result in life-threatening infections especially those who are carriers of resistant *S. aureus* strains (33). Different reports estimated that almost 25% of the health care workers are stable nasal carriers of *S.aureus* and they also represent reservoir of resistant factors to other pathogens and transfer this pathogens to those non carrier patients under their care (34–36). Medical students are those mostly in risk for an occupational exposure to MRSA during their clinical practice and also serve as bridge of transmission(37,38)

Methicillin resistant *S aureus* was mainly a problem in hospital-acquired infections(27). Over the past decade, community-acquired MRSA (CA-MRSA) has increased significantly in a number of countries.Fortunately, many of these CA- MRSA strains have so far retained susceptibility to a number of non-beta-lactam antimicrobials(29). Whereas most healthcare acquired MRSA (HA-MRSA) infections are caused by difficult-to-treat multi resistant strains and increases healthcare costs in addition to the new treatment options for MRSA also associated with problematic side-effects. Severe MRSA infections mostly occur during or soon after inpatient medical care and cause significant morbidity or mortality in health care settings(27,39–41).

Methicillin-resistant *Staphylococcus aureus* infection spread predominantly via person-to-person contact, contaminated surfaces and objects. Recurrent skin disease, and frequent antibiotic use, are common risk factors for MRSA colonization(8,9). Methicillin-resistant *Staphylococcus aureus* (MRSA) is probably the known example of resistant bacterium and has been the focus of intense scientific and political interest around the world(42). Screening of nasal carriage MRSA in HCWs is an important component in the control of MRSA in any healthcare facility. Identification of the colonized staff members allows an appropriate management to prevent the spread of organism within hospitals(43).

Because medical students belongs to the HCW, in future and Study on MRSA among medical students has not been frequently reported. Especially in sub-Saharan Africa as medical students interacting and exposed to not well structured and comfortable hospital environments in the future, We felt that medical students come into intimate contact with patients and hence may be an additional source of nosocomial infections in hospitals and they may be the potential nasal carriers and main agents for spreading the organism to hospitalized or hospital visiting patients, (44). Therefore the aim of our study is to determine nasal carriage rate of methicillin resistant *Staphylococcus aureus* among clinical year medical students. And the antimicrobial susceptibility as well as the contributing factors for MRSA nasal carriage.

1.3. Significance of the study

Nasal carriage rate of *S. aureus* and MRSA among healthcare personnel is an important source of HAI (15). Because clinical year medical students have prolonged contact with patients during hospital practice, they have greater exposure to MRSA, and more likely to spread MRSA to patients. In Ethiopia, a few studies have been conducted on prevalence of *S. aureus* and MRSA carriage in health care workers(45,46).But to the best of our knowledge, this is the first study to report nasal carriage rates of *S. aureus* and MRSA and its drug susceptibility pattern in clinical year medical students in Ethiopia. And there is no data on medical students who are thought to be risky groups as they practices in hospitals without well-developed clinical practicing skills.

Therefore, by identifying the rates of *S. aureus* and MRSA nasal carriage in clinical year medical students, this study aimed to show the burden and antimicrobial resistance pattern of MRSA in clinical year medical students, which helps to create an effective system for prevention nosocomial transmission of MRSA, and management of MRSA infection. Thus this study helps: As evidence-based information about the burden level of MRSA nasal carriage among clinical year medical students of Jimma University. The findings indicates groups at risk of colonization and this may help in targeting interventions to prevent transmission of the bacteria and Researchers also use this data as a base line data in identifying thematic areas on the matter for further study, show the evidence based gap for JUSH infection prevention office, Policy makers in designing appropriate strategies for preventive measures.

CHAPTER TWO: LITERATURE REVIEW

2.1 Biology of *staphylococcus aureus*

S. aureus is a Gram-positive spherical bacterium, with adiameter of 0.4-1.2μm and occurs in microscopic clusters resembling grapes. It can be distinguished from other *Staphylococcus* species by testing the coagulation of rabbit serum(47).

2.2. Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant *S.aureus* is a specific strain of the *S. aureus* which is defined by the presence of a large mobile genetic element called staphylococcal cassette chromosome (SCCmec). a gene, mecA, for methicillin resistance. The mecA gene codes for altered penicillin binding protein (PBP2a) which is different from the indigenous PBPs of *S. aureus*. PBP2a allows MRSA to continually synthesize its cell wall in the presence of β-lactam antibiotics(48). Methicillin, like all penicillins, exerts its action by blocking the proteins called penicillin binding protein (PBPs), which are responsible for the construction and maintenance of the bacterial cell wall. But *S. aureus* resistant strains acquired a new protein, called PBP2a, which is not blocked by methicillin and could replace the other PBPs, thus allowing the survival of *S. aureus* in the presence of methicillin(30). PBP2a and native PBP work in concert to allow cell wall synthesis despite the presence of beta lactam antibiotics. There are two distinct types of MRSA: The hospital acquired MRSA (HA MRSA) and community acquired MRSA (CA MRSA)(49).

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2.3. Epidemiology of MRSA

2.3.1 Global Epidemiology

MRSA can be found worldwide, Hospital associated strains tend to occur in all countries, although they can be rare in some areas e.g., some countries where eradication programs have been implemented.

Published reports on nasal carriage *S aureus* and MRSA show different patterns of epidemiology depending on difference in study subjects, exposure to hospital, and socio-economic status. Hospital acquired MRSA is prevalent in worldwide;approximately 4.6% medical workers worldwide are MRSA carriers. Data from 2004 and 2007 shows the prevalence of 54.2% and 58.1% in the USA, respectively(50). A review from 2011 describes a prevalence of hospital acquired methicillin-resistant Staphylococcus aureus was above 50 % in The United States. . Although still a common and severe threat to patients, the overall rates of invasive MRSA dropped by 31% in USA (27). MRSA proportions observed in Canada (in 2008) and Australia (in 2009) were 27.0% and 33.6%, respectively (43,51)

In Europe, MRSA proportions are generally lower in Northern Europe and higher inthe South and South-Eastern countries. The average proportion of MRSA isolated frominvasive infection was 17.8% in 2012 (52).Only two countriesreported proportions above 50%, which were Portugal (53.8%) and Romania (53.9). Themajority of the countries, 19 of 30, reported 10%-50%. Lower proportions were reported in sixcountries, all less than 3% including Denmark (1.3%), Finland (2.1%), Iceland (1.7%), theNetherlands (1.3%), Norway (1.3%) and Sweden (0.7%)(29,52,53)

In Asia Currently, more than 50% of *S. aureus* isolates show resistance to methicillin in most countries. Very high rates of MRSA were reported from East Asian countries, such as Korea (77.6%), Taiwan (65.0%), Hong Kong (56.8%), Thailand (57.0%), Vietnam (74.1%), but also Sri Lanka (86.5%),Iran reported 43.5% *S. aureus* isolates being MRSA. In contrast, much lower proportions were reported from India (22.6%) and the Philippines (38.1%) (51,52,).

Data of MRSA in Africa is limited however from available data; In African continent the first cases reported was in South Africa in 1978. A survey done in eight African countries between 1996 and 1997 showed a prevalence of MRSA was (21-30%) in Nigeria, Kenya and Cameroon(55). Since 2000MRSA was increasing in most of the countries. For instance; In Tunisia, increased from 16% to 41% between 2002–2007(55). While in Libya it was 31% in 2007. In Botswana, the prevalence varied from 23–44% between2000–2007. In Algeria and Egypt, the prevalence was 45% and 52% between2003–2005, respectively, In Ivory Coast, the prevalence was 39%,(25,41). Most countries had prevalence of 20% and above indicating the magnitude of the MRSA problem in the Africa Continent. In East African region, high prevalence of MRSA (31.5%) was found in surgical site infections in Mulago National Referral Hospital, Kampala Uganda(56). Furthermore, the implementation of infection control measures and the wide spread of HIV infection and tuberculosis, inadequate coverage of effective antibiotics and inaccurate antibiotics sensitivity tests done in the laboratories particularly in the sub-Saharan area; amplify the difficulty of dealing with MRSA epidemic in Africa(41).

2.3.2 Epidemiology in Ethiopia

A meta-analysis study conducted to determine pooled prevalence of MRSA in Ethiopia showed that the overall estimation of MRSA prevalence in Ethiopia was 32.5% (95% CI, 24.1 to 40.9% (31). Other studies on the prevalence of MRSA have been conducted in hospitalized patients and apparenthealthy individuals at different location showed different prevalence such as;from clinical specimen and nasal swabs of patients at Tikur Anbessa Specialized Hospital, Addis Ababa 68.0%(23);from Clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa17.5% (32); Mekelle Hospital health care workers,20.3% (45); Dessie Referral Hospital healthcare-workers 12.7%(46)from school children northern Ethiopia 13.8%(57);among primary school children and prisoners in Jimma, southwestern Ethiopia 23.0%(58)MRSA prevalence was found.

2.4. Risk factors

Differentstudies have investigated that the common determinants of MRSA colonization (carriage) are;

2.4.1: Hospital exposure;MRSA is most commonly found in hospitals, due to the fact that there are higher numbers of infected surfaces,equipments and patients in hospitals. Reports show hospital exposure increase colonization and infection of MRSA. For instance a comparative study done in Southern India showed that 9.2% of hospital exposed groups was colonized withMRSA, however from the non-exposed group, only 4% was MRSA carriers(59). Similarly on medical residents of Laval University Quebec; all residency levels from medical and surgical specialties and controls medical students without previous clinical rotations shows hospital exposed groups are more colonized(35).TheStudy in medical students of Taiwanese university, the carriage rate of MRSA was 16.8% for pre-clinical students and 21.9% for clinical students (60).

MRSA surveillance study done in 17 Asian hospitals in eight countries showed MRSA accounted for 25.5% of CA *S. aureus* infections and 67.4% of HA infections this shows high MRSA burden in hospitals(61).Similar study in Brazilian students on clinical posting are most likely at an increased risk of carriage than the pre-clinical (62)**.** Similar reportsfrom the University of Sarajevo, Bosnia medical students, (63). Korean students (64), In Medical Students of Colombia(65), In a Specialist Hospital in Saudi Arabia, (66) , in Brunei Darussalam(67), study in Namik Kemal University students(68).In Ethiopia (22,46,69).China(49)and in Cameroon (70).shows having hospital exposure increase risk of colonization as well as infection of MRSA.

2.4.2. Hand hygiene;Hand hygiene is considered the most important infection control measure in healthcare settingand forms the core for patient safetyandContaminated hands are considered the main vector of the spread of MRSA. Studies showed that alcohol-based antiseptic is more effective than non-medicated soap in reducing MRSA. studyconducted in Iraq showed that poor hygiene habits, close skin-to-skin contact is a contributing factor for MRSA colonization(12).Another study donein Nigeria showed MRSA and hand hygiene have significance association(71) .

2.4.3: **Repeated Antibiotic usage**

Antibiotics have been successful in treating bacterial infections. But, due to overuse of antibiotics, incomplete drug courses taken by infected individuals, or due to frequent antibiotic exposure which leads to increased opportunities for the development of resistant bacteria and due

to cross-transmission of resistant genes in addition to other reason, many clinically relevant bacteria have developed antibiotic resistance (72). In any large population of bacterial cells a few individual cells may spontaneously become resistant. Such "resistant" cells have no particular advantage in the absence of an antibiotic, but after treatment with antimicrobial agent, all sensitive bacterial cells will be killed, so that the initially very few resistant cells can proliferate and form a completely resistant population.This is supported by study in Iraq showed frequent antibiotic exposure is afactor for MRSA colonization(12) and Similarly study done in Republic of Korea confirmedthat most students who were colonized with MRSA had received antibiotics in the last 12 monthsof data collection(73).

2.5. Virulence factors of *S.aureus*

The remarkable ability of *S. aureus* to cause an enormous range of infections is due, in part, to its ability to produce multiple virulence factors. *S.aureus* can express proteins to bind fibrinogen, fibronectin, laminin, collagen, elastin and thrombospondin to promote adherence and attachment to endothelial cells and basement membranes. Collectively, these proteins are known as MSCRAMMs for microbial-surface components recognizing adhesive matrix molecules(1,18).

S.aureus also expresses Protein A, on its surface, which binds to the F portion of immunoglobulin, inhibits phagocytic engulfment and biochemical properties that enhance their survival in phagocytic cells. In stationary phase, *S.aureus* produces large numbers of membrane-damaging exotoxins and proteases to promote tissue damage. Tissue invasion is mediated by proteases, nucleases, lipases and staphylo kinase, a fibrin-specific thrombolytic enzyme. In addition, some toxemic strains of *S. aureus* produce super antigens, such as toxic shock syndrome toxin I (TSST-I), to activate large numbers of T cells resulting in proliferation and cytokine release(25,30,74).

2.6. Laboratory diagnostic methods

Laboratory screening for MRSA and *S aureus* is a complex balance between speed of result, sensitivity, specificity and cost.

2.6.1. Microscope

S. aureus (MRSA) are identified as Gram positive grapelike cocci in clusters showing deep violet color in gram stained smear.

2.6.2. Culture

Culture-based methods are still the backbone for *S.aureus* or MRSA detection. Detection of *S aureus* in mucocutaneous swab specimens is typically performed by using selective and differential agar media, sometimes enhanced with enrichment broth culture, the media contain an indicator system to presumptively identify *S. aureus,* such as mannitol and a pH indicator phenol red combined with inhibitory agents such as sodium chloride at high concentrations. And usually require 24–48hrs for identification (75). The Clinical and Laboratory Standards Institute (CLSI)and other guidelines recommends the cefoxitin disk screen test methods for MRSA detection(isolation)(7,76).

2.6.3: Biochemical:

Specific characteristic tests like the catalase test, the coagulase test and Commercial biochemical tests which use automated instruments can also be used to identify *S. aureus*.

2.6.4: Serological tests:

The latex agglutination tests for PBP2a provide results in 15 minutes with accuracy similar to that of a pure culture. Identification of toxins produced by *S. aureus*, such as enterotoxins A to D and TSST-1 in severe cases like toxic shock syndrome and food poisoning and Other tests are determined by clumping of the latex particles by the toxins present in the samples**.**

2.6.5: Molecular Methods

Molecular techniques including Real-time PCR and Quantitative PCR used in detection of the bacteria in real-time is being employed in clinical laboratories. TaqMan Real-time PCR methods is the most used method for detection of methicillin-resistant *Staphylococcus aureus* directly from screening specimens having sensitivity of 98% and specificity of 100% with Turnaround time(TAT) of 64hr. And SYBR Green, have sensitivity of 98% and specificity of 99% with Turnaround time (TAT) =2hr. (77,78)

2.7. Antimicrobial Resistance pattern

2.7.1. Resistance among *S aureus*

Antibiotic resistance of bacterial pathogens may vary according to exposure to antibiotics, rates of exchange resistant genes. In recent years, many S. aureus strains have acquired resistance to commonly used antibiotics. A study done on Indian medical students showed resistance to penicillin, cotrimoxazole, erythromycin, ciprofloxacin and tetracycline was found in (100%), (61.1%) (22.2%), (22.2%), and (16.6%), respectively(79**).** Another study on Brazilian medical students showed was colonized by *S. aureus*. Out of these *S.aureus* 86% were resistant to erythromycin, and 18.6% to clindamycin (62). Similarstudy done in Cameroon on medical staffs showed that most *S.aureus* strains were sensitive to clindamycin. Similarly study in Benghazi hospital HCWs showed resistance against penicillin and ampicillin (97.5%), (98.2%) respectively andthe lowest resistance was gentamycin (7.1%), clindamycin (7.5%) ciprofloxacin (3.2%)(80). Another study in Health Care Workers in Sa˜o Tome´ and Principe among the *S aureus* isolates, showed 36.6% resistance to trimethoprim-sulfamethoxazole, 12.2% to ciprofloxacin, 9.7% to tetracycline, and 4.9% to erythromycin. (19). Moreover, Similar report at Jimma prisoners showed that, among *S.aureus* isolates 100% were resistant to Ampicillin and Penicillin, 71.4% to tetracycline, 68.6.7% to erythromycin 57.1% to gentamicin and chloramphenicol each, , and 40% to co-trimoxazole(58).

2.7.2. Resistance among MRSA isolates

Strains that are resistant to methicillin are common and are designated methicillin resistant *S. aureus* (MRSA). Methicillin resistance is mediated by PBP-2a, a penicillin binding protein encoded by the mecA gene that permits the organism to grow and divide in the presence of methicillin and other β-lactam antibiotics. The mecA gene is located on a mobile genetic element called a staphylococcal chromosome cassette. The relative ease of transfer of this genetic element explains the growing resistance to β-lactam antibiotics such as penicillin and its chemical derivatives as well as the cephalosporin drugs(53).

The susceptibility of MRSA to various antibiotics varied among the studies. This can be differences in the type of the studied population and use of different antibiotics in different countries. Study inHCWs at national Medical College Teaching hospital, Birgunj Nepal, MRSA isolates were resistant to Ciprofloxacin (37.5%), Tetracycline (37.5%), (4).

Similar study in Cameroon on medical staffs showed that MRSA resistance was observed in trimethoprim/sulfamethoxazole(76%),followed by erythromycin (55%)(70). Study among Health Care Workers in Sa˜o Tome´ and Principe the MRSA isolates showed resistance to trimethoprim-sulfamethoxazole (85.7%), erythromycin (64.3%), tetracycline (57.1%), ciprofloxacin (42.8%)(19).Study in Dessie Referral Hospital which was conducted on health workers (HCWs) showed that among MRSA isolates, 73.3 % to tetracycline, 66.7% to co-trimoxazole, 46.7% to erythromycin, and 40%(46)

CHAPTER THREE: OBJECTIVES

3.1. General objective

- To determine nasal carriage rate of methicillin resistant *Staphylococcus aureus* among clinical year medical students of Jimma University, southwest Ethiopia.

3.2. Specific objective

- To determine the overall prevalence of nasal carriage *S aureus*and MRSAamong clinical year medical students.
- To assess contributing factors for colonization of MRSA in clinical year medical students
- To determine susceptibility pattern of *S.aureus*and MRSA.

CHAPTER FOUR: MATERIALS AND METHODS

4.1. Study area

The study was conducted at Jimma university specialized hospital (JUSH). JUSH is the oldest public hospital found in Jimma town. The town is located at about 352Km in southwest direction of Addis Ababa (the capital city of Ethiopia). JUSH is a teaching and referral hospital having about 400 beds with 1448 healthcare workers providing service for approximately 15,000 inpatients per-year within 11 wards, About 600 patients attending outpatient department daily. The hospital serves more than 15 million people living in the southwest Ethiopia catchment area. JUSH also a clinical practice center for JU health and medical students including health-officers, clinical nurses, midwifery nurses, Anesthesia, clinical pharmacy, medical laboratory, dental medicine and medical medicine under graduate students. Also give specialty training in diff medical disciplines i.e. (internal medicine, surgery, pediatrics, gynecology, ophthalmology and psychiatry. In this hospital about 750 clinical year medical students (C-I,CII and intern) are practicing in 2016 academic year (81).

4.2. Study Design and period

Institution based cross-sectional study conducted from May to August, 2016

4.3. Population

4.3.1 Source population

All clinical year health students who have beenattached to clinical practices atJUSH were considered as a source population.

4. 3.2. Study Population

All clinical year medical students who have been attached to clinical practices at JUSH during the study period were considered as the study population.

4.4. Eligibility

4.4.1. Inclusion criteria

Clinical year medical students who have been attached to clinical practices during data collection and those who were volunteer to participate in the study were included.

4.4.2. Exclusion criteria

- Clinical year Medical students who had taken antimicrobial agents in the last two weeks prior to data collection and those students with nasal infection and/or pathology during data collection were excluded.

4.5. Sample size determination and Sampling technique

4.5.1. Sample size determination

$$n = \frac{(Z\alpha/2)^2 P(1-P)}{d2}$$

n - Sample size

Z – 1.96 (at 95% confidence interval)

P –prevalence of MRSA= (p=12.7) (46)

d – Margin of error = ±5%

Substituting into the formula, n= 170, considering 10% non-response rate the final sample size was 187. To increase yields for relevant finding we were make it double i.e. =374

4.5.2. Sampling technique

Stratified sampling procedure was used to select study participants. The students in clinical practices at JUSH were stratified as clinical-I clinical-II, and medical-interns. We followed Proportional allocation method and the sample size of different strata were kept proportional to the sizes of the strata and the 1st student was randomly selected and then, Systematic sampling was used to select study participant in each stratum.

4.6. Study variables

4.6.1. Dependent variable

- ✓ nasal Carriage rate of Methicillin resistant *S.aureus* (MRSA)

4.6.2. Independent variables

- Sex
- Age
- Hand decontamination habits after patient care
- Using gloves during patient care
- Length of hospital practice
- history of repeated Antibiotic usage during the past one year

4.7. Data collection

4.7.1. Socio-demographic data and contributing factors

Socio-demographic, and potential risk factors like length of hospital exposure, hand washing habit, use of gloves whilehandling patients,and history of repeated antibiotic usage were collected by using structured questionnaires by investigator and trained health professional (2 BSc nurses) from May to August, 2016.

4.7.2. Laboratory data collection

4.7.2.1. Specimencollection

Swab samples were collected from each anterior nares with sterile cotton swabs by inserting cotton swabs approximately at 2-3 cm into one nares and rotating gently against the inner surface for 3-5 seconds and repeat this procedure to the 2nd nares using another cotton swab by the study participant themselves (Self-sampling) (82,83).Transferred to the bottles containing Amies transport medium (Oxiod, UK), which was labeled with code number, time and date. Swabs obtained were transported to Microbiology Laboratory of Jimma University using a cold

chainwithin 2 hours of collection and cultured immediately or placed at 4ºC refrigerators when delay for few hours is mandatory.

Figure 1: Nurses on data collectionin wards of JUSH, 2016

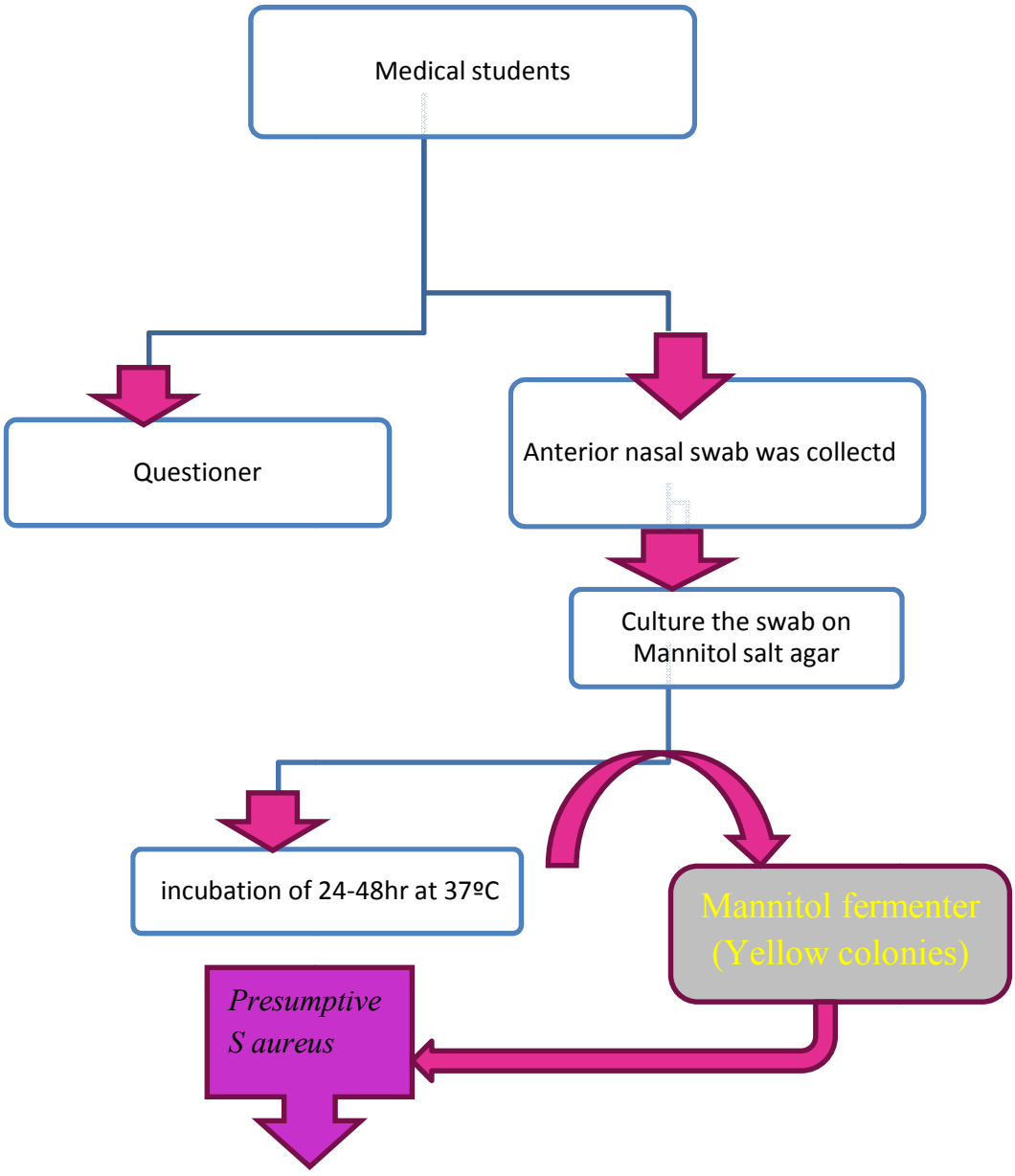
4.8: Culturing and isolation of *S aureus*

Specimens was plated onto mannitol-salt agar (Oxiod UK) and incubated at 37ºC for 24- 48 hours; Identification of *S. aureus* was based on mannitol-salt fermentation (golden or cream colored) colonies, colony morphology, gram stain, catalase test, and coagulase test. *S.aureus* isolates was incubated under-20ºC in tryptic soy broth with 15% glycerol until antibiotic susceptibility test was done.

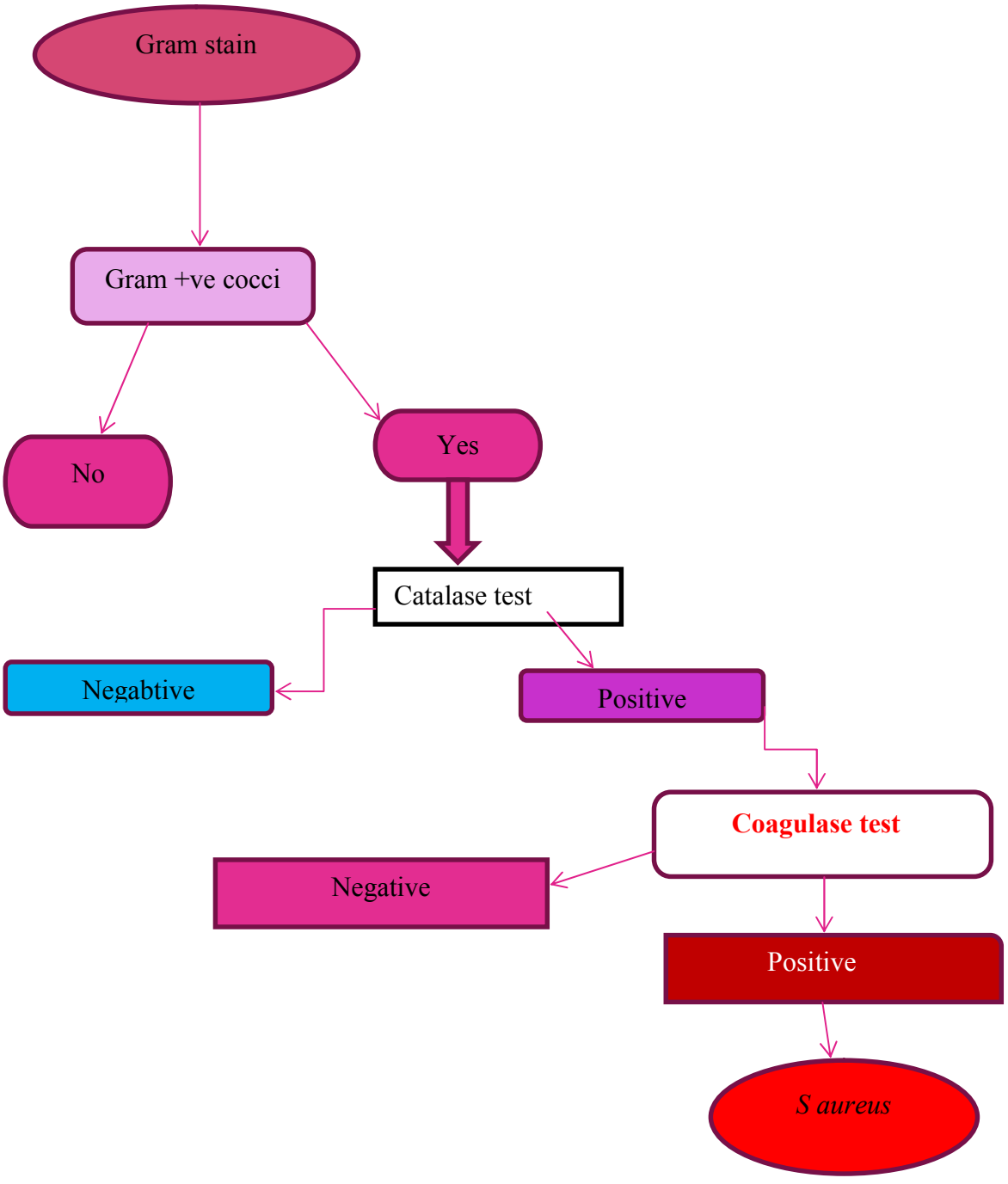
4.9: Identification of MRSA

Susceptibility of *S aureus* isolates to cefoxitin was tested to identify MRSA (84). All the isolates were subjected to Cefoxitin disc diffusion test using a 30µg disc. A 0.5 McFarland standard suspension isolates were made and inoculated on Mueller Hinton agar plate and then cefoxitin disk placed. Plates were incubated at 37ºC for 18–24 hours and inhibition zone diameters (mm) were measured. An inhibition zone diameter of ≤ 21mm was reported as methicillin/Cefoxitin resistant and ≥ 22mm was considered as methicillin /Cefoxitin sensitive.

Flow chart explaining the experimental work



To confirm the bacteria is *S. aureus:*



To identify MRSA from MSSA:

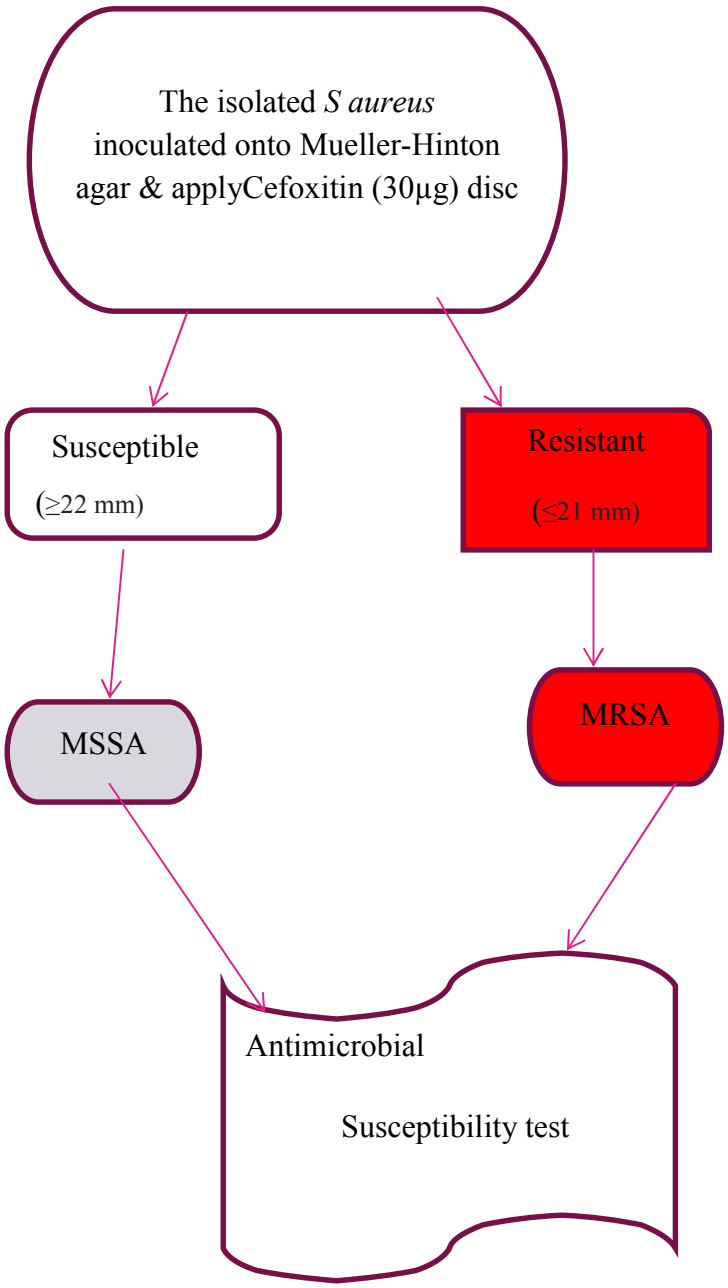


Figure 2: Flow chart of the experimental work

4.10: Susceptibility testing

Following identification of MRSA from MSSA,0.05 McFarland suspension of MRSA and MSSA was prepared and inoculated on Mueller-Hinton agar and then antimicrobial agents were placed on the surface of the inoculated agar as recommended by the clinical and Laboratory Standards Institute (CLSI)(84).Thefollowing antimicrobial agents weretested for MSSA; Penicillin G(10U),ciprofloxacin(5µg),clindamycin(2µg),gentamicin(10µg),erythromycin(15µg),chloramphenicol (30µg), ampicillin (10µg),Ceftriaxone (30µg), tetracycline (30 µg)and Trimethoprime-sulfamethaxazole (25 µg).*Staphylococcus aureus* ATCC 25923 control strains was used as control for MSSA isolates in order to check the potency of antimicrobial discs and to test the culture media. For MRSA isolates, antimicrobial agents that are commonly used to treat infections caused by MRSA were tested.These antimicrobials were clindamycin (2µg),erythromycin (15µg),trimethoprime-sulfamethaxazole (25 µg) andciprofloxacin (5µg).Attempts to test Vancomycin and carbapenems were not successful and some of the antimicrobial agents such as Oxazolidinones and streptogramins are not available at all.All intermediate readings were taken as resistant during data entry.

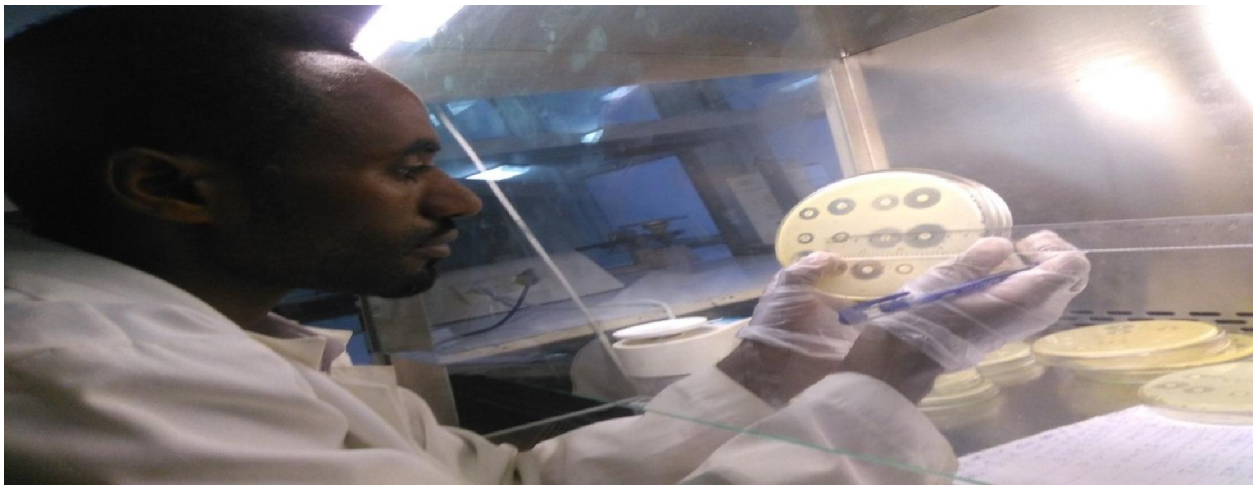


Figure 3: lab activities; measuring inhibition zones on Muller Hinton agar media

4.11: Data analysis

Data was edited, cleaned, entered and analyzed using statistical package for social science (SPSS) version 20. Descriptive analysis such as frequencies used. The chi-square test was employed to see whether there is statistically significant association between dependent and independent variables. P-value of < 0.05 was considered as statistically significant..

4.12: Quality assurance

Training was given to the data collectors on the objective of the study and each item on the questionnaires. Socio-demographic data and samples were collected by principal investigator and trained health professional nurses. Questionnaires were checked for their completeness regularly,andproblems encountered were discussed. Closer supervision was undertaken during data collection. Pre-analytical, analytical, & post-analytical quality control measures was carried out in all laboratory procedures. In all steps standard operational procedures (SOP) was followed andControl strain of *S aureus* ATCC 25923 was used to monitor the potency of antimicrobial discs and inoculating media.

4.13: Ethical consideration

The study was conducted after securing ethical clearance from ethical institutional Board (IRB) of health Institute of Jimma University. Official Permission paper was obtained from Jimma University specialized hospital. Similarly after clear discussion about the actual study or explaining the purpose of the study,written informed consent was obtained from each study participants, the study participant's right to refuse was respected. Confidentiality of test result of the participants was maintained and the test result used only for the research purposes. MRSA positive students was communicated and connected to senior internists.

4.14: Dissemination of a Study Finding

The findings of this study will be presented primarily on Master's thesis defense Final report will be submitted to Jimma University institute of Health, Faculty of Health Sciences, School of medical Laboratory Sciences, and also submitted to the Hospital administration. Moreover, the paper will be published on either national or an International Journal to communicate to the scientific community.

CHAPTER FIVE: RESULT

5.1. Overall prevalence of *S. aureus*

A total of 371medical students were enrolled. The response rate was 99.2%. Males were 315(84.9%) and 56 (15.1%) were females. Of these 82(22.1%) were found to be positive for *S aureus*. Among positive study subjects 69(18.6%) were males and the remaining 13(3.5%) were females. *S aureus* was most prevalent among medical interns 31(8.4%). The detail is presented by table-1

Table: 1: Distribution of *S aureus* by sex, age and year of study of Medical students who have been attached to clinical practices at JUSH, From May to August 2016, Jimma, Southwest Ethiopia.

		***S aureus* Positive N(%)**	***S aureus*Negative N(%)**	**Total**
Gender	Female	13(3.5%)	43 (11.6%)	56(15.1)
	Male	69(18.6%)	246 (66.3%)	315(84.9%)
Total		82(22.1%)	289 (77.9%)	371(100)
Age group	20-25	62(16.7%)	237(63.9%0	299(80.6%)
	26-30	20(5.4%)	52(14.0%)	72(19.4%)
Total		82(22.1%)	289(77.9%)	371(100)
***S aureus isolates* by year of study**				
Medical students		Positive N(%)	Negative N(%)	Total N(%)
Clinical-I		28 (7.5%)	138(37.2%)	166 (44.7%)
Clinical-II		23(6.2%)	102(27.5%)	125(33.7%)
Medical intern		31 (8.4%)	49(13.2%)	80(21.6%)
Total		82 (22.1%)	289(77.9%)	371(100)

5.2. Prevalence of MRSA

Among 82 *S aureus* isolates 31(37.8%) were found to be methicillin resistant *S aureus*. Whereas the remaining 51(62.2%) were found to be methicillin sensitive. This study revealed that the overall prevalence of nasal carriage MRSA to be 8.4% (31/371). MRSA were more prevalent among male study subjects 28(34.1%) than female study subjects 3(3.9%).And Medical interns were most commonly colonized by MRSA, 16(19.5%). and percentage of MRSA rate with in age group 26-30 were 45%. The detail is presented on table-2.

Table.2: Distributions of MRSA among medical students who have been attached to clinical practices at JUSH from May-August 2016, Jimma, and Southwest Ethiopia.

Medical Students		**[1]MRSA N(%)**	**[2]MSSA N(%)**	**Total N (%)**
		(n=31)	(n=51)	N (%)
Age	20-25	22(26.8%)	40 (48.8.5%)	62 (75.6%)
	26-30	9(11.0%)	11 (13.4%)	20 (24.4%)
	Total	31(37.8%)	51(62.2%)	82(100%)
Gender	Male	28 (34.1%)	41(50.0%)	69 (84.1%)
	Female	3 (3.7%)	10 (12.2%)	13 (15.9%)
	Total	31(37.8%)	51(62.2%)	82 (100%)
Clinical-I		6(7.3%)	22(26.8%)	28(34.1%)
Clinical-II		9(11.0%)	14(17.1%)	23(28.0%)
[3]M-intern		16(19.5%)	15(18.3%)	31(37.8%)
Total		31(37.8%)	51(62.2%)	82(100%)

[1]MRSA=methicillin resistant *S aureus*
[2]MSSA = methicillin susceptible *S aureus*
[3]M-intern=medical intern

5.3 contributing factors for*S. aureus* and MRSA colonization

In the current study, contributing factors like history of repeated antibiotic usage, using gloves while handling a patient and hand decontamination habits were not significantly associated with nasal colonization of MRSA (p>0.05). However, length of hospital practice was found to be significantly associated with nasal colonization of MRSA i.e. (P=0.03). The detail is presented by Table-3

Table.3: Statistical analysis of contributing factors to nasal colonization with MRSA among medical students who have been attached to clinical practices at JUSH from May-August, 2016,Jimma southwest Ethiopia.

Contributing factors	Number	MRSA		X2	P. value
		POS N(%)	NEG N(%)		
Using gloves while handling a patient					
Yes	75	27(36.0)	48(64.0)	0.484	0.48
No	7	4(57.1)	3(42.9)		
Hands decontamination score					
High[a]	11	4 (36.4)	7(63.6)		
Moderate[b]	36	11(30.6)	25(69.4)	1.75	0.41
Low[c]	35	16(45.7)	19(54.3)		
Repeated antibiotics use in the past one year					
Yes	8	4(50)	4(50)	0.13	0.71
No	74	27(36.5)	47(63.5)		
length of hospital exposure					
<1year	27	5(18.5)	22(81.5)		
1-2years	24	10(41.7)	14(58.3)	6.93	0.03
>2years	31	16(51.6)	15(48.4)		

[a]*High*decontamination *score (always using sanitizers),*[b]*Moderate (always using water/soap or plus sanitizerssometimes)* [c]*Low (always using water only or water/soap sometimes)*

5.4. Antimicrobial susceptibility pattern of MSSAisolates

A total of 51 MSSA isolates were subjected to antimicrobial susceptibility test against antimicrobial agents. *S.aureus* isolates were almost 100% sensitive to clindamycin followed by ceftriaxone 96.1% sensitive and Chloramphenicol 94.1%. However, no isolate was sensitive to Penicillin and Ampicillin.ThedetailispresentedbyFigure-4

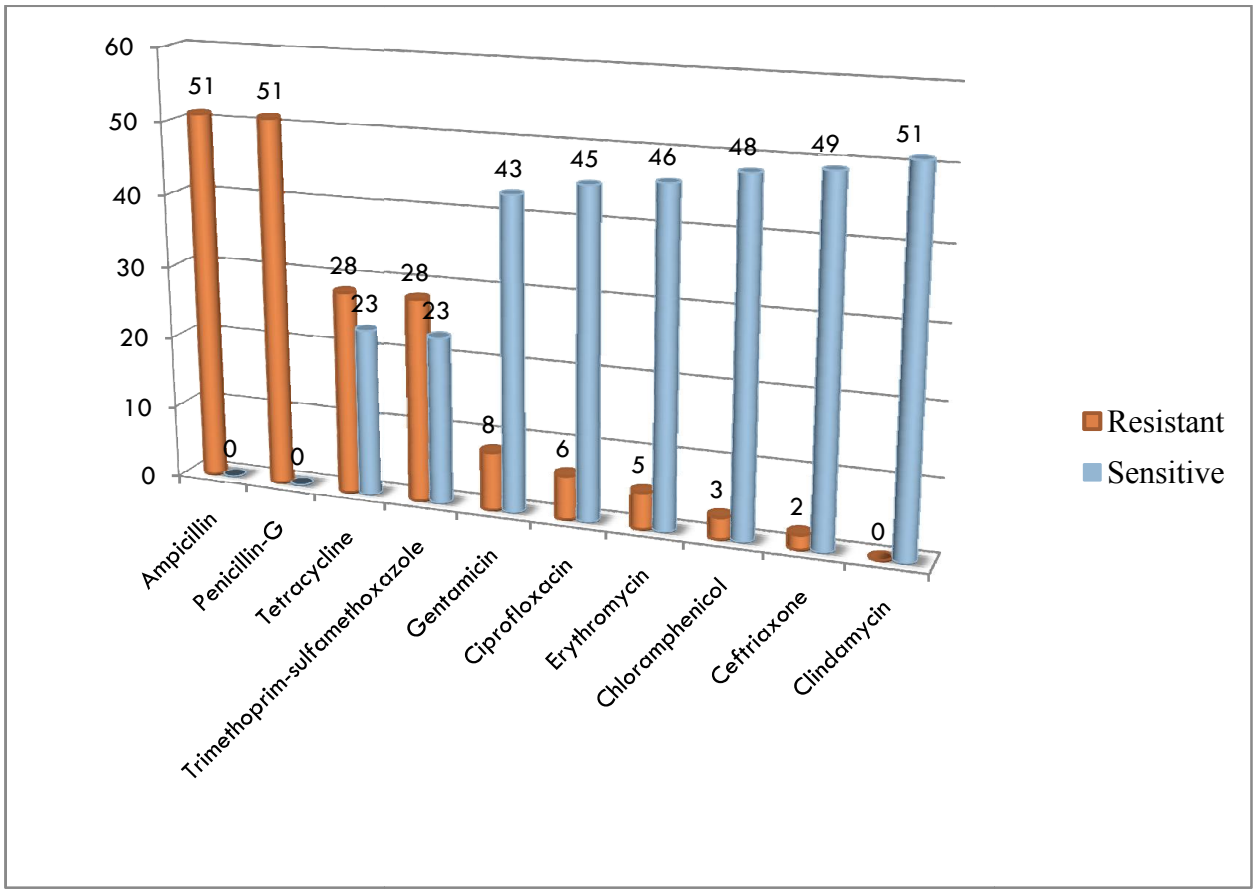


Figure 4: Susceptibility pattern of MSSA isolates, Jimma southwest Ethiopia May-August, 2016

5.5. **Antimicrobial susceptibility patterns of MRSA isolates**

Susceptibility of MRSA to antimicrobial agents that are commonly used to treat infections caused by MRSA was tasted. Unfortunately, the most effective drugs such as Vancomycin, Oxazolidinones, Streptogramins and Carbapenems were not included in this test. Clindamycin was found to be the most effective drug against MRSA. (83.9% sensitive) followed by Erythromycin (71.0.4% sensitive). The detail is presented by Table-5

Table 4: Susceptibility Patterns of MRSA to antimicrobial commonly used to treat infections caused by MRSA, Jimma southwest Ethiopia May-August, 2016.

Antimicrobial agents	**MRSA**	
	Resistant N (%)	Sensitive N (%)
Clindamycin(2µg)	5 (16.1)	26(83.9)
Erythromycin (15µg)	9(29.3)	22(71.0)
Ciprofloxacin(5µg)	16 (51.6)	4(48.4)
Tetracycline(30 µg)	20 (64.5)	11(35.5)
Trimethoprim-sulfamethoxazole(25 µg)	26 (83.9)	5(16.1)

5.6. Multi drug resistance pattern of MSSA isolates

Multi-drug resistance in this study was taken as resistance to three or more of the antimicrobial agents tested. Multidrug-resistant status of *S.aureus* isolates was tested against 9(nine) classes of antimicrobials agents. Among the total MSSA isolates;27/51(52.9%) isolates were multi-drug resistant. of them 15/27 (55.6%) was showed resistance to three different classis of antimicrobials and 9/27 (33.3%) isolates showed resistance to four antimicrobial agents and no isolates was fully susceptible to all the antimicrobial drugs. (Table: 6)

Table 5: distribution of multi drug resistance MSSA isolates among Jimma university medical studentsMay-August, 2016.

Year of study		**Frequency of resistant isolates**
		Total N (%)
Resistant to 3 drugs	AM,TE,SXT AM,SXT,GM AM, E, SXT AM,E,TE AM,TE,GM	8(29.6) 2(7.4) 2(7.4) 2(7.4) 1(3.7)
Resistant to 4 drugs	AM,TE,SXT,CHL AM,TE,SXT,CIP AM,TE,SXT,GM AM,E,TE,SXT AM,CRO,GM,CIP AM,COR,TE,SXT	2(7.4) 2(7.4) 2(7.4) 1(3.7) 1(3.7) 1(3.7)
Resistance to 5 drugs	AM,TE,SXT,GM,CIP AM,TE,SXT,CIP,CHL	2(7.4) 1(3.7)
Total		27 (100)

N.B:DA=clindamycin, E=erythromycin, SXT=sulfamethoxazole-trimethoprim, GM=gentamicin, TE=tetracycline, AM=ampicillin, CIP=ciprofloxacin, CRO=ceftriaxone, CHL = Chloramphenicol

5.7: Multi drug resistance pattern of MRSA

Among MRSA isolates; 15(48.4%) isolates were multi-drug resistant. of them 9/15 (60.0%) was isolates from medical intern students and 5/15 (33.3%) was isolates from clinical-I students. And no MRSA isolates was fully susceptible to all the five selected antimicrobial drugs**.**

Table 6: Resistance pattern of MRSA isolatesfrom Jimma university medical students to five selected antimicrobial agents May-August, 2016.

Year of study	**Antibiogram pattern**						**Total**
	R3	N (%)	R4	N (%)	R5	N (%)	N (%)
Clinical-I	DA, E, SXT	1(6.7)	DA, E, TE,SXT	1(6.7)	DA, E, TE,SXT,CIP	1(6.7)	5(33.3)
	E, TE, SXT	1(6.7)	E,TE,SXT,CIP	1(6.7)	_		
Clinical-II	TE,SXT,CIP	1(6.7)	_		_	_	1(6.7)
Medical intern	SXT,CIP,TE	6(40.0)	SXT,E,CIP,DA	1(6.7)	SXT,E,CIP, DA, TE	1(6.7)	9(60.0)
	SXT,CIP,E	1(6.7)	_		_		
Total	10(66.7)		3(20.0)		2(13.3)		15(100)

N.B:DA=clindamycin, E=erythromycin, SXT=sulfamethoxazole-trimethoprim,TE=tetracycline, CIP=ciprofloxacin

CHAPTER SIX: DISCUSSION

In this study the overall prevalence of nasal carriage *S aureus* and MRSA among clinical year medical students were 22.1% (82/371) and 8.4% (31/371) respectively. The carriage *rate* of *S aureus* in this study showed similarity with study done in medical students of Poland (22.4%)(85),Taiwan (21.9%) (86), Brazil (21.1%)(87) and Tanzanian (22.3%) (88). However it is lower than similar study done in medical students of Colombia 29.2% and Korea 28.8% respectively(34,73). Differences of rates between the different countries and hospitals may be explained by geographical areas, methodology used, study time and seasons of the year in which the studies carried out.

Based on their clinical year (study year) the highest*S. aureus* colonizedwere medical interns8.4%, followed by clinical-II 6.2% and (7.5%) clinical–I students.Thisin line with study in Saud Arabia medical students(89). M V J Medical College & Research Hospital in Bangalore, India(90).This may be due to Clinical-I and Clinical-II students had limited exposure to patients during their clinical practices, than medical interns Therefore, it is necessary to refresh students on infection prevention with in a given time intervals.

Overall MRSA carriage rate of thisstudy was 8.4%(31/371) and itis comparable with study done on Saudi Arabia medical students 6.7% (89), Nepal Medical students 10% (4), Ankara University Hospital Medical staff 9.1% and healthcare workers at the Kenyatta national hospital 9.5% (91). The compatibility is because of method similarity and the hospital infrastructure and facilities nearly similar because of all are in developing countries.

However, results of this study showed higher rates than other studies in different areas of the world such as Brazil medical students 3.2%(92), medical students of Iraq 4.6%(85),Medical studentsand healthcare workers ofwest Bengal,India2.9% (93), healthcare workers and Medical students of Democratic Republic of Congo 2.6% (94). The variation could be attributable to the strict adherence to the rules of disinfection and antisepsis by the medical students attending clinical practices and the comprehensive hygienic precautions taken by the infection control committee of the hospitals, rate of patient admission as well as different infection control and prevention policies across countries. Moreover, the high prevalence of MRSA nasal carriage

students participated in this study may be due to the fact that patients and students were overcrowded in this hospital.

The carriage rate of MRSA among medical student in our study was less than that of among health care workers inLibya21.4% (5)Northern regions of Ethiopia Dessie Referral Hospital 12.7% (46) and health care workers in Mekelle Hospital,14.1% (45). This may be due to hospital exposure of healthcare workers is longer than students.

Regarding to the factors contributing for*S aureus* and MRSA colonization, different factors including student related, health care worker related, and patient related and environmental related factors have been reported in different studies. In this study having longer hospital stay was prone to MRSA colonization; we found that the years of clinical exposure can affect the carriage rate of MRSA among medical students. The highest carriage rate 51.6% (16/31 MRSA was seen in medical interns (6^{th} year) students who were spent more than 2years in the hospital practices and the least MRSA carriage rate 19.4% (6/31) was in clinical-I (4^{th} –year) Medical students who were spent less than one year in clinical practice. These results were compatible with previous studies from Brazil, Taiwan, Saud Arabia and Darussalam Medical students respectively(86,87,89,95). The possible explanation for the high prevalence of MRSA among medical intern students could be due to the long hospital stay on average 60hr/week,close contacts with the hospitalized patients, because of work load and/or due to adaptation, interns becomes reluctant to follow infection prevention procedures like glove usage, hand washing aftercares. During data collection we witnessed that C-I students were more adhered to basic infection prevention precautions, after contact patients than C-II and Medical interns.

Regarding age of the medical students in the present study identified that being elder (26-30 years) as a factor to the MRSA colonization. The reason behind is because of most students of this age groups were students who spent many years in clinical practices and due to seniority they are more expected to diagnose patients and work load, exhaustion may cause negligence to follow infection prevention precautions. This finding is in line with study done in Medical Faculty of Turkey University and M V J Medical College & Research Hospital, Dandupalya, India respectively (90,96).

Medical students who didn't use gloves while handling patients had higher carrier rate (57.1%) of MRSA compared with those that used gloves (36%). those medical students with the highest sterilization score (who were using a sanitizer always) had the lower carriage rate of MRSA 4(36.4%) than students who were use alcohol based sanitizer sometimes(moderate sterilization score) 15(44.1%). This implies that using sanitizer (alcohol based hand rub) is more impactful in reducing carriage of MRSA even it was not statistically significant. There is few literatures which in line with this finding like study done on Iraq patients and medical students (12) and Nigerian tertiary hospital health care workers(71)

In this study being male was more prone to MRSA colonization than females and this finding is similar with study done in medical students at a Taiwanese university, Taiwan(60). The probable explanation for this could be in our study participants gender is not proportional and female students are more adhered than males on infection prevention precautions, and other additional factors may cause the differences.

Antimicrobial resistance is one of the vital microbial threats in the twenty-first century. Surveillance on the antimicrobial susceptibility patterns of *S. aureus* is extremely important in understanding new and emerging resistance trends. In this study different antibiotic susceptibility patterns were observed. Among the resistant pathogens, MRSA is of great concern because of its particular importance in causing various clinical conditions(97).

Antimicrobial susceptibility test of MSSAisolates against commonly used antibiotics indicated that almost all isolates (100%) were resistant topenicillin and ampicillin. This is comparable with resistance detected in Indian medical students(79), Benghazi hospital HCWs(80), Egypt(98)and Jimma prisoners (58), furthermore, the isolates also showed resistance rate of 54.9%, to Trimethoprim-sulfamethoxazole and Tetracycline each, 15.7% toGentamycin and11.8%,toCiprofloxacin respectively.This is in line with study done on Health Care Workers in Sa˜o Tome´ and Principe(19)and Dessie referral hospital health care workers (99) .

On the other hand, the MSSA isolates were sensitive to clindamycin 100% followed by Ceftriaxone (96.1%), Chloramphenicol (94.1.%),Erythromycin (90.2%),which is comparable with corresponding study of Iraq healthcare workers and Tikur Anbessa Specialized Hospital(23).

In this study MRSA isolates were relatively increased resistanceto, Trimethoprim-sulfamethoxazole (83.9%), tetracycline (64.5%) and Ciprofloxacin(51.6%).The higher resistance of the isolates against these antibiotics commonly used to treat MRSA infections is might be due to continuous genetic variation (mecA) of the strains by mutation of an existing gene, or horizontal transfer of a resistance gene from another bacterium and alteration of PBP2A.Andin additioninaccurate diagnosis and isolation of pathogens in the laboratories, leading to the overuse or misuse and inappropriate use of antibiotics.Resistance to TMP-SMX in MRSA seems very high this is common inother African countries, TMP-SMX resistance rates range between 23–100 % for MRSA(94). Prophylactic use of TMP-SMX in human immunodeficiency virus (HIV) patients may impact resistance.

While clindamycin showed a lower resistance result (16.1%) followed by Erythromycin (29.3%). This result was in accordance with study conducted in Congo(94)

Multi-drug resistance in this study was taken as resistance to three or more classes of the antimicrobial drugs tested. From MSSA isolates 27/51(52.9%) were multi-drug resistantand 15/31(48.4%) of MRSA isolates was resistant to three and more antimicrobial agents out of five selected drugs which are commonly used to treat infections caused by methicillin resistant *Staphylococcus aureus* (MRSA).This is comparable with 54.8% Multidrug resistance in CameroonMedical staffs (100) and higher than The Hospital Nacional Cayetano Heredia, Peru 25%(11)and less than85.8% in Egypt (98) andmedical staff of the Yaoundé University Teaching Hospital, Cameroon 76.0%(101). The possible factors for the difference may be diversity in local infection control practices, regional differences in antibiotic availability and prescribing behavior may have an influence.

This study did not investigate vancomycin resistance situationamongisolates; but our results clearly indicated that there is high multi drug resistance in MRSA and MSSA isolates and this isrisky for the students themselves, their colleaguesas well as patients under their care. In view of these results, we propose that further investigationamong health students who have hospital attachment for clinical practices should be carried out to know their role in MRSA spread.

LIMITATIONS AND STRENGTH OF THE STUDY

- The strength of this study was, maximizing sample size bymade double the calculated sample size to increase yields for relevant finding
- It was not possible to conduct identification of different resistant genes like mecA and PVL-toxin which would have provided us distribution of strains and the extent of antibiotic susceptibility patterns in the area.
- The colonization was due to community or hospital acquired strains could not be identified
- Unavailability of some important drugs like Vancomycin,Oxazolidinones and Streptogramins

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusions;

In this study,the prevalence of MRSA among medical students at Jimma University current setting was higher as compared toprevious studies done among clinical year medical students in fewAfrican countries. We conclude that asclinical exposure increases, there is aconsistentincrease in the Methicillin resistant *Staphylococcus aureus nasal carriage*.Even though it was not statistically significant, use gloves while handling the patients and using alcohol based hand rub after patient diagnosis decrease the rate of colonization.As MRSA is the most common nosocomial pathogen, the clinical year medical students have the potential to transmit to the patients during their hospital practice, and at the same time they are also at a higher risk of carrying the pathogen themselves.

The susceptibility test results showed that almost all isolates of MSSA were resistant to penicillin and ampicillin. However, Chloramphenicol, Ceftriaxone and Clindamycin showed highsensitivity rates to MSSA isolates. The MRSA isolates showed high sensitivity to clindamycin 83.9(%) and Erythromycin (77.6%). and this two drugs could be used as treatment. Additionally this study showedthat the overall Multi-drug resistance detected in 52.9% of MSSA isolates and 48.4%in MRSA isolates

7.2. Recommendation

- Screening for resistant strains of MRSA among clinical year medical students should be adopted as a protocol in university hospitals in order to control the spread of MRSA with in the hospitals and from the hospitals to community.
- Periodic training of medical student on infection prevention is needed in order to reduce the nosocomial spread of MRSA.
- Alcohol-based hand rubantisepticsshould beplaced at every bedside and/orstrategicallyplacedin thehospitals.
- soap and clean water accessibility should be improved in the hospitals
- Clindamycin is the recommended drug for both MRSA &MSSA isolates
- Further studies are needed to clarify the role of medical students in spread of MRSA in hospital environment and transmission to the patients.

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Annexes

JIMMA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

ANNEX I: Information Sheet

Introduction: This information sheet is prepared by groups of researchers whose main aim is to study.Nasal carriage methicillin resistant *Staphylococcus aureus* among clinical year medical students in Jimma university, south west, Ethiopia The investigators include a second year Microbiology student, and advisors from Jimma University, college of health sciences, Department of Medical Laboratory Sciences and Pathology.

Purpose:

The purpose of this research is to determine nasal carriage rate of methicillin resistant *staphylococcus aureus* among clinical year medical students in Jimma university, studies in different countries have reported MRSA carriage rate increase in health care facilities especially on health care workers and students in clinical. However, in our country, and as well as in JUSH, no study done on clinical year medical students and the magnitude of MRSA is Unknown. Therefore, considering nasal carriage methicillin resistant *staphylococcus aureus*, we have planned to undertake the research among JU clinical year medical students.

Procedure:

We kindly invite you to take part in this project which is aimed at determining the nasal carriage methicillin resistant *Staphylococcus aureus*, among clinical year students. as mentioned earlier. If you are willing to participate in this project, you need to understand and sign the agreement form. You will be asked to fill some questions associated with risk factors for colonization of S aureus and, you will provide nasal swab sample collected from the nares. The swab samples will be collected following a standard protocol. The laboratory examination results will be kept confidential using coding system whereby no one will have access to your laboratory results. If the result of the laboratory examination shows positive for MRSA, this will only be communicated to you and the medical specialists

Risk and Discomfort

During swab sample collection you not feel any pain and discomfort, which will be followed closely, sterile cotton applicator stick will be used and there is no need to worry about acquisition of any pathogens.

Benefits

If you participate in this research, you may get direct benefit that the test result will be used for yourself, you know your carriage status and based on the decision of the physician you will treated if you will positive for MRSA. In addition, your participation will help us in determining nasal carriage methicillin resistant *staphylococcus aureus* among clinical year medical students in Jimma University, south west, Ethiopia which is an input to design control strategies of colonization and infection of MRSA, especially hospital setup.

Incentives

You will not be provided any incentives to take part in this research.

Confidentiality:

The information that we collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. I will not be revealed to anyone except the principal investigator and the physician for your benefit.

Right to refuse or withdraw

You have full right to refuse from participating in this research if you do not wish to participate or to withdraw in the meantime but your input has great value for the success of our objective. And. The study has no risk to you except mild time consuming Therefore I politely request your cooperation to participate in this study.

ANNEX II: Consent Form

I have been informed about a study. For this study I have beenrequested to give nasal swab sample from the nares. I have been read all the information stated in the introductory part and I have had an opportunity to ask any ambiguous question I got satisfactory answer for all of my concerns. I have fully understood and gave my consent to give the swab specimen. It is therefore, with full understanding of the situation that I gave my informed consent and cooperate at my will in the course of the conduct of the study.

Informed Consent number_____

Participant code----------Signature ----------Date ----------

Name (data collector) ----------Signature ----------Date ---------

Thank you for your cooperation!!!

ANNEX:III; Questionnaire:

Part I - Sociodemographic characteristics of respondents

No	Questions	Response category code	
01	Sex of the respondent	Male ---------- ----- Female----------	
02	Age		

Part II-Risk factors associated with MRSA

N_o	Questions	Response category	
03	Year of study	Clinical –I..…..…… ☐ Clinical -II…… ☐ Medical intern…….…… ☐	
04	How long time is it since you started clinical practice?	by month _____ . by year _____	
05	Average days of hospital practice per week?	_____ days	
06	Average hours of hospital practice per day	_____hrs.	
07	Did you use gloves while handling a patient	Yes_____ No	
08	Is there hand washing facility in your unit/ward?	Yes_____ No _____	

09	Frequency of hand washing after patient contact a) With soap and water b) with water only	Always _____ Sometimes _____ Rarely _____ No _____	
10	Do you cleaning your hand by use hand rub antiseptic	yes _____ if yes how often Always _____Sometimes _____Rarely_____ No _____	
11	Did you have taken antibiotics within two weeks back?	yes _____ No _____	

ANNEX- IV; Lab. data collection format

LABORATORY RESULTS FOMAT:

Lab code _____			
Lab tests	Positive	Negative	Remark
1. Mannitol salt fermentation			
2. Gram's stain			
3. Coagulase test			
4. Catalase test			
5. Cefoxitin susceptibility test			

ANNEX-V

Media Preparation, Procedure for Specimen Collection and Processing

Amies transport medium

1. The preparation was as instruction of the manufacturer.

2. Sterilize by autoclaving at 121ºC for 15min

3. The medium was cooled, and tightened when the bottle caps. The bottles were inverted during cooling, to ensure an even distribution of the charcoal.

4. The medium was dated and given it a batch number.

5. The medium was stored in a cool place away from direct light. Shelf life up to nine month.PH within the range of 7.1 -7.3 at room temperature

6. The specimen was collected on a sterile cotton wool swab and immerses it in the medium, cutting off the swab sticks to allow the bottle top to be replaced tightly. Protect the swab from direct light and heat.

Mannitol Salt Agar (MSA)

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

Preparation of mannitol salt agar

1. Measure 1000ml of distilled water and add into a conical flask.

2. Weigh 111g of Mannitol salt agar powder.

3. Add and suspend the measured MSA into the 1000ml of distilled water.

4. Heat with frequent agitation and boil for one minute to completely dissolve the powder.

5. Autoclave at 121°C for 15 minutes.

6. Cool to 45-50°C for dispense

7. Arrange the petri-dishes onto the clean safety hood and then gently pour (18-20ml) onto the plates.

8. Cover the petri-dishes and allow the media to coagulate before storage in a refrigerator.

9. Label on the bottom of the plates name of media, preparation date and expiration date and store at 2-8c

10. Every batch prepared media was quality controlled for both sterility and the ability to support growth of target organisms

Mueller Hinton Agar (MHA)

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

organism. Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive results for pathogenic microorganisms.

Preparation of Mueller Hinton Agar

1. Measure 1000ml of distilled water into a conical flask.

2. Weigh 21g of muellerhinton agar powder.

3. Add and suspend the measured powder into the 1000ml of distilled water. Mix thoroughly.

4. Heat with frequent agitation and boil until completely dissolve the powder.

5. Sterilize by autoclave at 121°C for 15 minutes and cool to 45-50°C overnight

6. Arrange the petri-dishes onto the clean safety hood and then gently pour the media onto the plates.

7. Test the sterility by incubating some media at 37ºC for 24hrs

8. Label with name of media, preparation date, expire date, and store at 2-8ºC for maximum two months

Colony characteristics and morphology

Preliminary identification of bacterial isolates was done using colony morphology and characteristics. After overnight growth each nasal swab sample was streaked (in duplicates) into mannitol salt. Then the plates were incubated aerobically at 37 0C for 24 hours and a control strain S. aureus ATCC 25923 was also streaked separately for confirmation. Those isolates which fermented mannitol salt agar with yellow color appearance were selected, then transferred into tryptone soya broth and incubated at 370 C for 24 hour. Again the samples were streaked into general purpose media, nutrient agar to get pure colony of the isolate. The characteristic isolates obtained were further identified using standard microbiological methods which included Gram‘s staining reaction and biochemical tests.

Gram Stain

I. Principle The gram stain is used to differentiate gram-positive and gram-negative bacteria. Cellular morphology can also be determined. Gram-positive and gram-negative bacteria are both stained by crystal violet. The addition of iodine forms a complex within the cell wall. Addition of a decolorizer removes the stain from gram-negative organisms due to their increased lipid content. These cells are stained pink with the counter stain safranin.

II. Specimen The gram stain can be performed on the growth of any strain grown on any type of media.

III. Reagents and Material

1. Crystal Violet Stain

2. Gram Iodine

3. Decolorizer Solution

4. Methanol

5. Slides

6. Inoculating loop

7. Microscope with Immersion Objective

IV. Procedure

1. Smears were prepared from cultures by emulsifying a part of colony in a drop of normal saline Spread over 1/3 to 1/2 the total area of a clean glass slides.

2. The smear was allowed to air dry

3. Cover the entire bacterial smear with 3 or 4 drops of methanol or passing over the flame to fix the smear and allow to dry

4. The bacterial smear was covered with crystal violet stain and allows standing for 1 minute. Gently washed with cool tap water and drain water from slide.

5. The smear was covered with grams iodine and allows standing for 1 minute. Gently wash the

iodine off with water and drain the water from the slide.

6. The smear was rinsed with decolorizer solution for 10 seconds; decolonization was completed when the solution runs clear from the slide. Gently rinse with water and drain the slide.

7. The smear was covered with safranin stain, and allowed to stand for 1 minute, then gently wash the stain from the slide.

8. The slide was blotted dry with absorbent paper and the slide was examined under oil immersion lens.

V. Reading and Interpretation The gram stain is used to aid in the differentiation of the gram positive cocci. The arrangement of the cells divides on random planes form grape-like clusters of cells

Biochemical testing procedures

Catalase Test

I. Principal Hydrogen peroxide is used (H_2O_2) to determine if bacteria produce the enzyme catalase.

II. Specimen Culture growth on a blood free media or colony growth on blood agar plate carefully transfers in to a slide without carry-over of any of the erythrocytes. Culture growth was typically seen at 35ºC-37 ºC for 24hrs in CO_2.

III. Reagents and Materials

1. Three percent hydrogen peroxide was obtained from a commercial drug store.
2. Pipette
3. Slides

IV. Procedure

1.The catalase test was best performed by very carefully picking the center of the 24 hour pure fresh

culture colony from a Mannitol salt agar plate with a the help of sterile inoculating needle or wooden applicator stick and transferring the colony to a glass slide. 2-3drop of 3% hydrogen peroxide was added to the colony and mixed.

V. Reading and Interpretation Any sign of bubbling will be interpreted as a positive test. The absence of bubbling was interpreted as negative.

The Tube Coagulase Test

Principle:

This method helps to measure free coagulase. The free coagulase secreted by *S.aureus* reacts with coagulase reacting factor (CRF) in plasma to form a complex, which is thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma.

Procedure:

1. Three test tubes are taken and labeled “test”, “negative control” and “positive control”.

2. Each tube is filled with 1 ml of 1 in 10 diluted rabbit plasma.

3. To the tube labeled test, 0.2 ml of overnight broth culture of test

4. Bacteriais added.

5. To the tube labeled positive control, 0.2 ml of overnight broth culture of known *S.aureus* is added

6.To the tube labeled negative control, 0.2ml of sterile broth is added.

7. All the tubes are incubated at 37oC and observe the suspensions at hourly intervals for a periodof four hours.

8. Positive result is indicated by gelling of the plasma, which remains in place even after inverting thetube.

9. If the test remains negative until four hours at 37oC, the tube is kept at room temperature for overnight incubation.

Procedures for antimicrobial susceptibility testing

i. Suspend colonies from a fresh plated culture into sterile phosphate buffer saline solution and adjust the density of suspension to that of a 0.5 McFarland standard.

ii. Use a sterile cotton swab (squeeze the swab against the side of test tube to remove the excess fluid) and evenly inoculate the bacterial suspension on the entire surface of Muller-Hinton agar.

iii. Apply the antibiotic discs after drying the plates for 3-5 minutes.

iv. Incubate the plate aerobically at 35-37ºC for 18-24 hours.

v. Measure the zone of inhibition and interpret according to breakpoints of disk diffusion method.

ANNEX-VI. Declaration

The undersigned declare that this M.Sc. thesis is my original work, has not been presented for degree in this or any other university and that all source of materials used for the thesis have been fully acknowledged.

Name:_____

signature:_____

Date:_____

This thesis has been submitted with my approval as University advisor

First advisor:

Name:_____

signature:_____

Date:_____

second advisor :

Name:_____

signature:_____

Date:_____

APPROVAL SHEET OF THESIS

As a member of the board of examiners of the master of science thesis open defense examination, I certify that I have read, evaluate the thesis prepared by Feyissa Efa, and examined the candidates as well. I recommended that the thesis be accepted by fulfilling the thesis requirements for the degree of Master of Science in Medical microbiology.

1. Internal Examiner_____

   Date_____ Signature_____

2. External Examiner_____

   Date_____ Signature_____