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Nasal and Hand Carriage Rate of Methicilin Resistant *Staphylococcus aureus* (MRSA) Among Health Care Workers in Mekelle Hospitals

By

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Masters of Science in Medical Microbiology.

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Table of contents

Contents **page**

List of Tables **page**

Abbreviations

ARH	Ayder Referral Hospital
CA-MRSA	Community Acquired Methicillin Resistance *Staphylococcus aureus*
CNS	Coagulase Negative Staphylococcus
CPS	Coagulase Positive Staphylococcus
GNB	Gram Negative Bacilli
HA-MRSA	Hospital Acquired Methicillin Resistance *Staphylococcus aureus*
HCW -	Health Care Workers
MDRO	Multi Drug Resistance Organisms
MH	Mekelle Hospital
MRSA	Methicillin Resistance *Staphylococcus aureus*
PVL -	Panton-Valentine Leucocidin (PVL)
S.aureus	*Staphylococcus aureus*
SCC	Staphylococcal Chromosomal Cassette
SSSS	Staphylococcal Scaled Skin Syndrome
TSS	Toxic Shock Syndrome
VRSA	Vancomycin Resistance *S.aureus*

Abstract:

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is the most significant pathogen responsible for hospital and community based infections that ranges from mild skin infection to serious and invasive disease such as pneumonia, septicemia. Nosocomial infections due to MRSA are a known cause of increased hospital stay, cost, morbidity and mortality especially among the critically ill patients. In most hospitals of developing countries like Ethiopia there is no surveillance system for nasal and hand MRSA carriage among HCWs. So, the aim of this study was to assess the carriage of MRSA in HCW.

Methods: A cross sectional study between Nov/2010-Jan/2011 was carried out to screen all the health care workers (HCW) in all wards of the Ayder Referral Hospital (ARH) and Mekelle Hospital (MH) for MRSA nasal and hand carriage rate. Swabs of both anterior nares and finger web of the hands were taken, transported to regional laboratory. The samples were inoculated onto Mannitol Salt agar (MSA) and incubated aerobically at 37^0C for 48 hrs. *S. aureus* was identified as mannitol fermenter and coagulase test positive. Anti- microbial susceptibility test for MRSA was done by Kirby-Bauer's disk diffusion method using oxacillin disk.

Results: Out of the 177 Health Care Workers screened, 36(20.3 %) of them were MRSA carriers in their hand and anterior nares. Females 25(14.1%) were highly colonized by MRSA than males 11(6.21 %) (P=0.044, Odds ratio=1.41). Nasal carriage of MRSA 25(14.1 %) was higher than hand carriage 11(6.2 %) (P<0.05). Nurses 26(13.6 %) and medical doctors 4(2.3 %) were the most predominant carriers for MRSA. The isolated MRSA were multidrug resistant to other commonly available antibiotics. They were resistant to Ampicillin (88.9 %), Tetracycline (86.1%), Amoxicillin (75%), Chloramphenicol (58.3 %) and Cftriaxone(52.8 %). Only two (5.6%) of the nasal isolates were Vancomycin resistant.

Conclusion: MRSA carriage among HCWs in this study was high. The carriage rate was higher among nurses and doctors. The MRSA isolates were multi drug resistant to other antibiotics which may lead to increased morbidity and mortality if transmitted to critically-ill patients. So, the result of this study shows more emphasizes for the need of regular surveillance of HCWs.
It also calls a need for an effective infection prevention and control program.

Key words: Methicillin, Resistance, *Staphylococcus aureus*, Mekelle, Health care workers

Chapter 1.Introduction

1.1. General characteristics of Staphylococcus

Staphylococcus: Staphyle, meaning “bunch of grapes” are Gram positive cocci arranged in grape like irregular clusters and also found in single or in pairs. They are non motile, non-spore forming, facultative anaerobe, catalase positive, cocci of uniform size (1um in diameter) that grow readily in many types of media. They are metabolically active fermenting carbohydrates with the production of lactic acid without gas and produce different pigments. They are resistant to high temperature as high as 50^0C, drying and high salt concentration. The genus Staphylococcus has 41 species and sub species. *S. aureus, S. epidermidis, S. saprophyticus, S. haemolyticus* and *S. lugdunensis* are the main species medically important in this family .*S .aureus* is the most virulent species of the genus Staphylococcus (Makgotlho, 2009).

Methicillin resistant *Staphylococcus aureus* (MRSA) has become endemic worldwide since the past two to four decades in hospitals as well as in community called as Hospital Acquired-MRSA and Community Acquired-MRSA, respectively. MRSA together with vancomycin resistance *S. aureus* (VRSA) and extended spectrum beta-lactamase producing gram negative bacilli (GNB) are classified as multi drug resistance organisms (MDRO). Since 1959, treatment of S. *aureus* infections included semi-synthetic penicillin drugs such as methicillin. But, in 1960s MRSA strains appeared and it was identified in the early 1980s as major nosocomial infection (Azeez-Akande, 2010; Makgotlho, 2009).

The role of MRSA carriers in the transmission of this pathogen is serious. A carrier of MRSA is a person who is colonized by the organism in the anterior nares (nose), sputum, open -wound urine, stool or skin without clinical manifestations of disease. Such carriers many transmit the organism to another person via direct contact, usually through colonized hands and aerosolization following sneezing. Therefore, health care workers who are at the border between the hospital and the community may serve as agents of cross-transmission of HA-MRSA and CA-MRSA. Correspondingly, screening and eradication of MRSA from colonized health workers is an important part of a comprehensive infection control policy for the bacteria (Azeez-Akande, 2010; Makgotlho, 2009).

The carriage rate of MRSA and its antibiotic resistance to other drugs is increasing in HCWs and becoming worldwide problem (Akande, 2010, Albrich and Harbarth, 2008).

Though MRSA causes many complication, has rapid transmission from one person to person and is a challenge for patient treatment due to high antibiotics resistance, there is scarcity of published data in HCWs in many developing countries like Ethiopia. Therefore, the aim of this study was to assess the nasal and hand carriage MRSA in HCW in Mekelle, Tigray, Ethiopia.

Chapter 2: Literature review

2.1. Morphology and characteristics of *S. aureus*

S. aureus is the most medically important species of the genus Staphylococcus. It is Gram positive cocci, occurs as single, pair or cluster, catalase- positive a distinguishing character from streptococci, coagulase-positive which distinguishes from other coagulase negative Staphylococci, DNAse positive and ferments glucose forming acid without gas. It ferments mannitol distinguishing characteristic from *S. epidermidis*. It is facultative anaerobes, grows in blood agar forming B- hemolytic colonies and form golden/yellow colonics in Mannitol Salt agar (MSA). The bacteria form part of the normal flora of the skin, intestine, upper respiratory tract and vagina. The ability of resisting high salt concentration and drying enables *S. aureus* to colonize the skin and nose (Makgotlho, 2009).

Table 2.1. Classification of *S. aureus* (Makgotlho, 2009).

Domain	Bacteria
Kingdom	Eu-bacteira
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	Staphylococcus
Species(of human importance)	*S. aureus, S. saprophyticus, S.epidermidis, S.lugdunensis*

2.2. Pathogenesis and virulence of *S.aureus* infections

S. aureus is an important human pathogen. It turns out to be pathogenic when circumstances such as PH, Temperature and nutrient availability are altered and become favorable for its growth. The pathogenicity of *S. aureus* is determined by its ability to produce extra cellular structures, different enzymes and many toxins like the 33kd protein alpha toxin, exfoliatin A and B and Panton-Velantin Leukocidin (PVL). These virulence factors are harm full to the

host causing diseases ranging from minor skin disease to deep infections like, meningitis and pneumonia (Makgotlho, 2009).

S. aureus causes many infections with the following clinical spectrum of disease: Skin and soft tissue: Impetigo, boils, carbuncles, abscesses, cellulitis, fasciitis, pyomyositis, surgical and traumatic wound infections. Foreign body associated: Intravascular catheter, urinary catheter, surgical implant, endotracheal tubes. Intravascular: Bacteraemia, sepsis, septic thrombophlebitis, infective. Bone and joints: Septic osteomyelitis, septic arthritis. Respiratory: Pneumonia, sinusitis, otitis media. Invasive infections: Meningitis, surgical space infection. Toxin mediated diseases: Staphylococcal toxic shock, food poisoning by releasing enterotoxin , Staphylococcal scalded skin syndrome due to exfoliative toxin produced by phage II strains of *S. aureus* (Braunwald *et al, 2008*; Matouskova and Janout, 2008; Zechovsky, 2000; Kluytmans, 2009).

Table 2.2 Virulence factors of *S. aureus* for its pathogenesis (Makgotlho, 2009).

Toxin and Enzymes	**Activity**
Haemolysins	Cytolytic: lyses erythrocytes of various animal species
Coagulase	Clots plasma, also used in clinical microbiology laboratories to differentiate between *S. aureus* and CNS
Fibrinolysin	Digest fibrin
Leukocidin	Kills leukocytes
Hyaluronidase	Breaks down hyaluronic acid
DNAse	Hydrolyses DNA
Protein A	Lypolytic (produces capacity in egg-yolk medium, binds to Fc portion of IgG
Capsule(some strains)	Anti phagocytic
Super antigens	Rheumatoid arthritis, diabetes mellitus, toxic shock syndrome
Epidermolytic toxins A and B	Epidermal splitting and exfoliation
Enterotoxin (s)	Food poisoning toxins that causes vomiting, diarrhea, general malaise without fever.
Toxic shock syndrome toxin-1	Shock, rash, rapid diarrhea, vomiting, fever, muscle pain

2.3. Epidemiology of *S .aureus* infection

S. aureus is part of the normal flora where the anterior nares is the most common site of human colonization though other sites are also colonized. Approximately, 25-50% of healthy persons may be persistently or temporarily colonized with *S. aureus.* For the future *S. aureus* infections, colonized persons are at greater risk than those non-colonized. Both higher rates of *S. aureus* nasal carriage and high subsequent rates of infection have been associated with many underlying diseases or conditions including insulin-dependent diabetes mellitus, long-term dialysis, children, intravenous drug abuse, repeated injections for allergies, liver cirrhosis, liver transplant, human immunodeficiency virus infection, long time hospitalization and surgical patients. Higher *S. aureus* infection rates are associated with activities leading to skin lesions such as contact sports. The common factor between these conditions is due to the repeated violation of the skin or mucosa as anatomical barriers (Belkum *et al,* 2005; Makgotlho, 2009).

The transmission of *S. aureus* in hospitals is often as a result of exposure to patients and health care workers who are *S. aureus* carriers. Treatment of *S. aureus* has become difficult due to the ability of the bacteria to develop multi-drug resistance. Methicillin resistant *Staphylococcus aureus* (MRSA) has become worldwide problem since the past two to four decades, and it occurs in hospitals as well as in community called as HA-MRSA and CA-MRSA, respectively. MRSA together with vancomycin resistance *S. aureus* (VRSA) and extended spectrum beta- lactamase producing gram negative bacilli (GNB) are classified as multidrug resistance organisms (MDRO). In the 1940s the choice of treatment for *S. aureus* infection was penicillin but two years latter *S. aureus* strains that resist for penicillin appears. Since 1959, treatment of *S. aureus* infections included semis-synthetic penicillin drugs such as methicillin. However, by the end of 1960s MRSA strains were appeared and it was identified in the early 1980s as major nosocomial infection (Balc *et al,* 2009; Azeez-Akande, 2010; Makgotlho, 2009; Sampathkumar, 2007). Mortality, morbidity, long hospital and treatment costs of MRSA infections have all increased year by year (Balc *et al,* 2009).

Table 2.3. Time line of *S. aureus* infection and resistance (Sampathkumar, 2007).

Year	Event
1940	Penicillin introduced
1942	Penicillin resistant *S. aureus* appears
1959	Methicillin introduced
1961	MRSA appears
1963	First hospital outbreak of MRSA
1968	First MRSA in USA was isolated
1970	Clonally spread of MRSA globally, high rate in Europe
1982	4% MRSA in USA was seen
1980-1990	Dramatic decrease in MRSA in N. Europe
1996	VRSA reported in Japan
1997	Approximately 25 % MRSA rate in USA hospitals vancomycin use increases VISA appears serious CA-MRSA infections reported
2002	First clinical infection with VRSA in USA
2003	MRSA increases by 60 % in ICU, out breaks of CA-MRSA seen
2006	CA-MRSA rate increases.
2007	Called as the year of" MRSA". Surveillance in 2004-2005 estimates: 95,000 invasive MRSA infections 19,000 deaths from MRSA per year. Continued report in CA-MRSA. Several states pass legislations regarding control of MRSA. Staph and MRSA becomes house hold words

Health care workers who are at the interface between the community and hospital may be the source of MRSA infection by carrying the bacteria in their body. Three types of MRSA carrier status can be distinguished for health-care workers:

They could be (1) non-carriers (2) persistent carriers; who are chronically colonized with the same strain and (3) intermittent or transient carriers, who are colonized with varying strains for short time periods (Albrich and Harbarth, 2008).

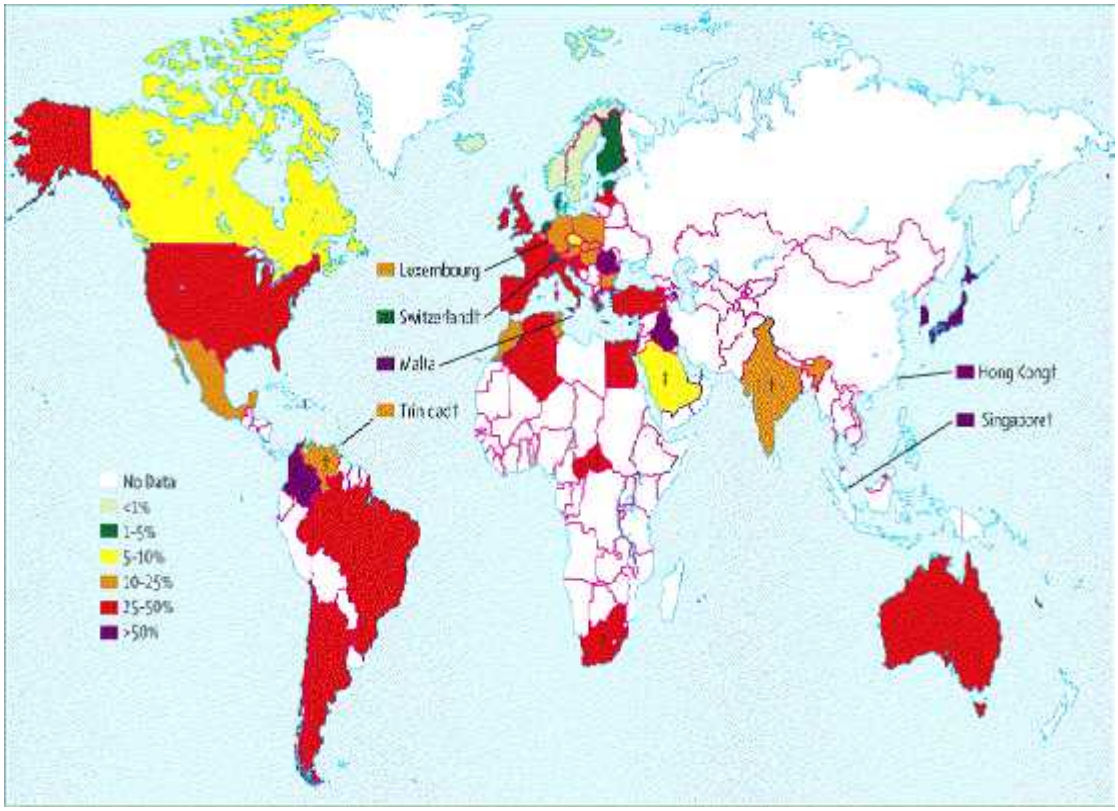

Figure 2.1 Worldwide Prevalence of MRSA (Alberta *et al*, 2008)

It was reported a prevalence of MRSA > (50 %) in Singapore, Japan and Colombia while countries with MRSA prevalence of (25-50 %) includes Mexico, Australia, S. Africa and the lowest prevalence of MRSA < (1 %) was reported from Norway, Sweden and Iceland (Alberta *et al*, 2008). A study conducted in 2007 reported that the prevalence of MRSA was more than (50 %) in Cyprus, Jordan and Malta which may be attributed to the overcrowding and poor hand hygiene facility in the hospitals (Makgotlho, 2009). In a review of Meta analysis study by (Albrich and Harbarth, 2007), it was reported that health care workers account for (93%) of personnel to patient transmission of MRSA.

A study conducted in Nigeria showed (52.5 %) prevalence of MRSA in nasal and hand of health care workers (Aderibigbe *et al*, 2010). Another study conducted in Ethiopia showed MRSA prevalence of (42.8 %) (Gabriel and Kebede, 2007).

A similar study on nasal carriage of MRSA in HCWs in teaching hospital of Nepal has reported a carriage rate of (10 %) (Mihreta *et al,* 2010). A research conducted in Taiwan in MRSA carriage, infection and transmission in dialysis patients, HCWs and their family members indicated a prevalence of (3 %) (Chang *et al*, 2010). Screening of HCWs in burn units of tertiary care hospitals in India for nasal MRSA colonization revealed (50 %) (Aravind *et al,* 1999).

Studies in MRSA transmission, the possible importance of un-recognized HCWs carriage from USA showed (6 %) (David *et al,* 2008) and similar study conducted in *S. aureus* nasal carriage among HCWs from Nepal hospital has reported (2.3 %) MRSA isolates (Mohaparta *et al,* 2009). Other studies from Iran in nasal colonization of HCWs by MRSA showed (5.3 %) a prevalence of (Alborzic *et al*, 2009). MRSA carriage rate of (14.3 %) in HCWs was reported from Turkey (Baki *et al,* 2009). A research in antimicrobial resistant bacteria among HCWs in intense care unit (ICU) at Ainshaz university hospitals, Cairo showed nasal and hand carriage rates of (22 %) (Abdel Rhaman, 2010).

2.4. Genetics and the development of anti biotic resistance

2.4.1. Oprational Definition

Resistance is the ability of a micro organism to develop a mechanism not to be in activated or killed by an anti biotic.

Methicillin is semi synthetic penicillin which is used to treat beta-lactamase producing bacteria that developed resistance to natural penicillin.

MRSA is by definition any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics which include the semi synthetic penicillins (methicillin, dicloxacillin, nafcillin, oxacillin) and the cephalosporins.

MRSA carrier is a person colonized with MRSA either transiently or permanently in his parts of the body with out clinical manifestation and who could be a potential transmitter.

MRSA strains carry *mecA* gene that encodes Penicillin Binding Protein2a (PBP2a), and it is carried by a mobile genetic element designated Staphylococcal cassette chromosome *mec* (SCC*mec*). There are five (I-V) SCC*mec* types where I, II, III and sometimes IV have been mostly linked to hospital associated MRSA strains and IV and V associated with community-associated MRSA strains (Azeez-Akande, 2010; Kluytmans and Struelens, 2009).

Table 2.4. Characteristics of HA-MRSA and CA-MRSA (Kluytmans and Struelens, 2009).

Characteristic	Hospital associated MRSA	Community associated MRSA
Patients characteristics	Older age underlying diseases common	Younger age underlying diseases rare
Specific groups at risk	Patients in hospital or other health care workers	Athletes, military recruits, children attending day care centers, prisoners, men who have sex with men, native Americans, native Australians and people in contact with living pigs.
Spectrum of disease	Bacteraemia, surgical site infection, pneumonia and urinary tract infection	Skin and soft tissue infection (such as abscesses, cellulitis, folliculitis, impetigo) and necrotizing pneumonia
SCCmec types	I, II, III and sometimes IV	IV and V
Antibiotic susceptibility	Multidrug resistant	Susceptible to most non-β lactam antibiotics
PVL	Rare	Common

Key: SCC= Staphylococcal cassette chromosome, PVL=Panton-Valentine leucocidin

2.5. Risk factors for MRSA infection

The following results in high risk of MRSA infection: People with low innate immunity, direct contact with an infected or colonized individual, crowded and unhygienic living conditions, recent long-term antibiotic use, history of frequent antibiotic use, individuals with skin wound, *S. aureus* colonized individuals, individual with dermatitis and pharyngitis, those who frequently visits outpatient (including outpatient surgical procedures), patients with surgery, catheter dwelling, percutaneous medical device, injection or intravenous drug users, those who share clothing and/or equipment and other items, underlying chronic illness like HIV/AIDS, people with diabetes, chronic renal infection, hypertension, contact with family member or household working in health care facility, care taker for person with unknown history of MRSA infections, patients undergoing dialysis, liver transplantation, getting in touch with bed linens of an infected individual, those living in close proximity such like in prisons, dormitories, army barracks, child care settings and long time hospitalization (Belkum *et al* ,2005; Azeez-Akande, 2010; Makgotlho, 2009; Matouskova and Janout, 2008; Aroutcheva *et al*, 2010). Increasing age, male

sex, alcoholism, lung disease and cancers have also been reported as risk factors for MRSA colonization (Belkum *et al*, 2005).

2.6. Laboratory diagnosis of MRSA

Diagnosis of MRSA from specimens can be diagnosed using the following methods.

I. Culture methods: MRSA like other *S. aureus* grows on Mannitol Salt agar (MSA) or blood agar with beta- hemolytic colonies at 35-37 C^0 for 24-48 hrs aerobically and micro aerobically. The colonies become visible yellow in MSA and white in blood agar. Isolated colonies are further identified by catalase test which is positive, coagulase positive and DNAse positive.

II. Antimicrobial susceptibility testing: MRSA strains are specifically diagnosed by anti microbial susceptibility testing using methicillin or oxacillin disks by Kirby-Bauer disk diffusion methods. In fact, this method has its own limitations due to the heterogeneity of the different strains which some of them may be methicillin sensitive *S. aureus* (MSSA) and some of them may be MRSA in a single culture media. These methods are inexpensive; however, time-consuming and standardization is difficult (Balc *et al*, 2009).

III. Serology techniques: using commercially available latex agglutination assay kits.

IV. Molecular techniques: These techniques display the highest level of sensitivity and allow simultaneous detection of *S. aureus* and the *mecA* gene (Balc *et al*, 2009; Makgotlho, 2009).

2.7. Prevention and treatment of MRSA infection and its spread

2.7.1. Treatment of MRSA infection

Generally, *S. aureus* infection is treated using penicillin if the strain is sensitive. Cephalosporins such as Cefazolin can be used for penicillin allergic patients. Patients infected with beta-lactamase producing *S. aureus* are treated by semi synthetic penicillin like: Methicillin, Oxacillin, Naficillin and Cloxacillin, and those infected with MRSA are treated by glycopeptides called Vancomycin and Teicoplanin. Tetracycline, Ciprofloxacin, Clindamycin and Co-trimoxazole are also indicated for Vancomycin intolerable patients. Novel quinnollones like Ciprofloxacin which have high anti S*taphylococcal* activity are not advisable for treatment due to the rapid development of resistance. Other new drugs like oritavanin and tigecyctine are under evaluation. So, appropriate treatment of *S. aureus* infection using drug sensitivity method is very

important for management of the MRSA occurrence (Makgotlho, 2009; Kluytmans and Struelens, 2009).

2.7.2. Vaccine for *S.aureus*

Yet, there is no approved vaccine that stimulates active immunity against *S. aureus* infection in humans. ***Staph*vax** which is composed of *S. aureus* type 5 and 8 capsular polysaccharide conjugated to non toxic recombinant *P. aeroginsa* exotoxin A is currently under investigation as a vaccine for *S. aureus* infection (Makgotlho, 2009).

2.7.3. Prevention of MRSA occurrence and its spreading

Occurrence and spreading MRSA infections can be prevented by: Patient screening before admitting to critical wards, HCW screening, treatment of carriers using topical application of mupirocin nasal cream and washing with disinfecting agents such as chlorhexidine, using isolation rooms and a barrier procedure, cleaning and decontamination of the equipments and the environment (Kluytmans and Struelens, 2009; Balc *et al,* 2009; Azeez-Akande, 2010; Aderibigbe *et al*, 2010).

2.8. Significance of the Study

MRSA is one of the most significant pathogen responsible for nosocomial infections. Mortality and morbidity rates in MRSA infections is roughly twice the level of those seen in cases of methicillin sensitive *S. aureus* (MSSA) infections and the cost of treatment for MRSA is 1.5 - 3 fold that of MSSA (Balc *et al*, 2009).

MRSA infection has become endemic problem of the world both in hospitals and community and its control has become a serious concern since the last two and four decades. MRSA associated infections created a serious burden in terms of medical and socio-economic costs and causes significant morbidity and mortality (Fauci *et al*, 2008). Report of active population based surveillance conducted in 2004-2005 estimated 95,000 invasive MRSA infections and 19,000 deaths from MRSA per year (Sampathkumar, 2007).

Update information about the distribution of MRSA in HCW is important to prevent the spread of HA-MRSA to community. Because once it spreads to the community it is not feasible to control due to asymptomatic carriers (Aderibigbe *et al*, 2010; Janout and Matouskova, 2008). Studies from developing and developed countries showed high carriage rate of MRSA in health care workers (Albrich and Harbarth, 2008; Janout and Matouskova, 2008; Gabriel and Kebede, 2007; Alborzic *et al*, 2009; Abdel Rhaman, 2010; Baki *et al*, 2009; Fadeyi, 2010).

MRSA infection is high among chronic patients like renal disease, HIV and hypertension liver cirrhosis. HIV positive patients have chance of getting the MRSA is six fold than those HIV negative (Aroutcheva *et al*, 2010). Nasal carriage of MRSA in healthcare workers is asymptomatic and early diagnosis of this carriage is extremely advantageous to preventing transmission (Balc *et al*, 2009). So, health care workers who are at the interface between the community and the hospital may be source of infection to patients, their family and ultimately to the community.

There is scarcity of published data on MRSA carriage rate in HCWs in Ethiopia. So, the aim of this study was to assess the prevalence for appropriate management of MRSA infection.

Most of the studies done so far are mainly in nasal carriage rate of MRSA and that of hand carriage seem to be overlooked; though they are an important vehicle to transmit the bacteria (Albrich and Harbarth,2008; Aderibigbe *et al*, 2010; Janout and Matouskova, 2008).

In addition to methicillin and other semi synthetic penicillin, *S. aureus* has developed resistance for other commonly used antibiotics which is a challenge for treatment of patients (Azeez-Akande, 2010; Aderibigbe *et al*, 2010; Makgotlho, 2009).

Update information in antibiotic susceptibility pattern of MRSA to other drugs is paramount since accurate empirical treatment of *S. aureus* infection is one of the most important step towards the reduction of the development of resistance in the different strains of *S. aureus* (Aderibigbe *et al*, 2010).

The above mentioned reports have tried to point out that the problem due to MRSA is very high, indicating that more attention needs to be given. Therefore, this study was undertaken to assess the prevalence of MRSA and its antibiotic susceptibility pattern to other commonly used antibiotics in health care workers at Mekelle hospital and Ayder referral hospital, Mekelle, Tigray, Ethiopia. The results were compared with the previous studies done in Ethiopia and elsewhere in the world. Findings from this study will also provide update information for appropriate management of MRSA infections.

Chapter 3. Objective of the Study

3.1 General objective:

- To assess nasal and hand carriage of MRSA among HCWs in Mekelle, Tigray, Ethiopia.

3.2 Specific objectives

- To determine the prevalence of MRSA in nose and hand of health care workers in Mekelle Hospital and Ayder Referral Hospital
- To determine the drug sensitivity pattern of the isolated MRSA to other commonly used antibiotics.
- To assess possible associated risk factors of MRSA in HCWs.
- To generate base line information for further study.

Chapter 4: Materials and methods

4.1. Study Design and period:

Cross sectional study was conducted from November 24-2010 - January 16, /2011 to assess the nasal and hand carriage rate of MRSA in HCWs.

4.2. Study area: The study was conducted in Ayder Referral Hospital and Mekelle Hospital in Northern Ethiopia, Tigray, Mekelle. Mekelle town which is founded in the 13th century is the capital city of Tigray region. It is located 787 km North of Addis Ababa. The total population is about 258.258 million and its elevation is 2,084m above sea level. The two largest ethnic groups in this town are the Tigrayan (96.5 %) and the Amhara (1.59%). Tigringa is spoken as a first language by (96.26 %) and (2.98 %) speak Amharic. Majority of the population (91.31 %) practiced Ethiopian Orthodox Christianity, and 7.66 % are Muslim and the remaining practiced other religions (Central Statistics Agency (CSA) National statistics, 2007). The town has one referral hospital, one general hospital and four health centers.

Mekelle Hospital, the largest general hospital in Tigray, is giving community service for Afar, Northern Amara, and Tigray regions since the last fifty years. Currently, it has 184 HCWs with different specialties/work categories and 320 beds in the different wards. Annually it gives medical service for about 125,300 patients. Ayder Referral Hospital (ARH) is a ground plus five teaching referral hospital of Mekelle University in the Health College Sciences which started community service since 2006. It owes 500 beds in the different wards. It has 217 HCWs and it gives service for an average of 29,153 patients annually.

4.3. Source population:

The source populations were all health care workers (HCWs) in the two hospitals.

4.4. Study Participants:

Volunteer HCWs who are working in Mekelle hospital and Ayder referral hospital, Mekelle, Tigray, Ethiopia were included in the study. Demographic data like age, sex, profession, year of services in the hospitals, duration of work in the units/wards, history of skin, nose infection, and antibiotic usage and other information were collected using structured questioner prepared for this purpose (annex.1).

4.5. Sample Size determination:

The sample size (n) was calculated by taking the prevalence of nasal carriage rate of MRSA in HCWs done in Ethiopia which was (42.8%) (Gabriel and Kebede, 2007). The expected margin of error (d) was 0.05 and the confidence interval (Zά/2) was 95%.

$$n = \frac{(Z\alpha 2)^2 * P(1-P)}{d^2}, \frac{(1.96)^2 * .428(.572)}{(0.05)2} = 376$$

since the source of population is < 10,000 ,the correction furmula is used .

$$\frac{n}{1+n/N} = \frac{376}{1+376/401} = 177$$

4.6. Sampling methods: A convenient sampling method was used to recruit the study participants.

4.7. Eligibility criteria

4.7.1. Inclusion criteria: All HCWS who were working in the different wards for two and more years were included in this study.

4.7.2. Exclusion criteria: All non health care workers were not participating in the study.

- ♥ HCWs who work in the hospitals for less than two years were not included in this study.

4.8. Variables

4.8.1. Dependent variables

- ✓ MRSA carriage rate
- ✓ Anti biotic susceptibility pattern of MRSA

4.8.2. Independent variables

Age, sex, duration of work in single ward, work category/professional, ward /unit type, history of skin disease, current anti biotic use and history diabetes of are independent variables.

4.9. Sample Collection, Handling and Transport

A total of 354 swabs from both anterior nares and webs of both hands were collected from 177 health care workers after getting consent during the period of Nov 24/2010 - Jan 16 /2011. Nasal specimens were collected by commercial sterile cotton swabs (NASATO, India) moistened with normal saline (0.85% NaCl). The swab was carefully inserted into each nostril so that its tip is entirely at the nasal osteum level (about 2.5 cm from the edge of the nares) and rubbing the swab four times around the inside of nostril by applying an even pressure and rotating the swab without interruption. Another swab was used to collect from both hand fingers. The swabs then were kept in Amies Transport media (Oxoid, England, UK) to maintain the viability of microorganisms until the specimen is processed. The specimens were transported within one to two hours to the regional laboratory for processing.

4.10. Sample Processing and Bacterial Identification

Samples were inoculated on Mannitol Salt agar (MSA) immediately after they reach the regional laboratory using the standard method and incubated aerobically at 37 ^{0}C for 48 hrs. A control *S. aureus* strain was also inoculated in another MSA with every batch of samples. Coagulase test using slide method was done after sub culturing of pure colonies to nutrient broth. Those colonies which were mannitol fermenter (golden or yellow colonies), coagulase positive were taken as *S. aureus*. Whereas, those with a white colony (mannitol non fermenters) and coagulase negative were considered as other *Staphylococci species.*

4.11. Antimicrobial Susceptibility Testing

Colonies confirmed to be *S. aureus* were taken and inoculated in to two separate Muller Hinton agars for sensitivity test of methicillin and other nine commonly prescribed antibiotics according to Kirby- Bauer disk-diffusion technique. Sensitivity test for methicilln was done using oxacillin disk (1ug) (Oxoid). The plates for oxacillin and the other nine drugs were incubated at 33^{0}C for 24hours and 37^{0}C for 24-48 hours, respectively. Finally, oxacillin resistant *S. aureus* isolates were checked for their sensitivity pattern against the other drugs in the other pate. Procedures were done according to the standard table supplied by the NCCLs (Wayne, 2000).

Three to five pure colonies were selected and transferred to a tube containing 5 ml peptone water and mixed gently until a homogenous suspension was formed and incubated at 37^{0}C until the turbidity of the suspension become adjusted to a McFarland 0.5. A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton Plate (Oxoid). The inoculated plates were left at room temperature to dry for 3-5 minutes.

The drugs for disc diffusion testing of the oxacillin resistant *S. aureus* were in the following concentrations: Ampicilin (AMP) (10µg), Amoxicillin-Clavulanic Acid (AMC) (30µg), Ceftriaxone (CRO) (30µg), Chloramphenicol (C) (30µg), Erythromycin (E) (15µg), Gentamicin (CN) (10µg), Vancomycin (V) (30µg), Tetracycline (TE) (30ug) and Amoxycillin (AML) (2ug) all (Oxoid). Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using an electronic digital caliper and the isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by the NCCLs (Wayne,

2000; American Society Manual sec. edition (ASM). High, intermediate and low level of resistance is defined when the percentage of resistance is >80 %, 60-80 % and < 60 %, respectively.

Oxacillin was used in the place of methicillin and those strains of *S. aureus* which are referred to as MRSA are usually oxacillin resistant *S. aureus* (ORSA). Though methicillin and oxacillin are similar antibiotics, oxacilin has more potential to induce the *mecA* gene which is responsible for drug resistance than methicillin. However, because of its historic role, the acronym MRSA was preferred in this study (Gustavo *et al*, 2001; Cheesbrough, 2002).

4.12. Reference Strains

Methicillin sensitive *S. aureus* (ATCC-29213) donated by Ethiopian health and nutrition research institute (EHNRI) was used as a quality control throughout the study for antimicrobial susceptibility testing.

4.13. Statistical Analysis

Data entry and analysis was done using computer with the new version 16 of SPSS soft ware. Descriptive methods were used for calculation for the results of study subjects separately by sex, age, profession and wards/units. Comparisons were made using Chi-square test with Fisher exact tests. P-value of <0.05 was considered indicative of a statistically significant difference.

4.14. Ethical Consideration

Ethical approval was obtained from ethical committee of the department of Microbiology, Immunology, and Parasitology of the School of Medicine, Addis Ababa University, before the study. Official permission from the study sites was obtained. Written informed consent was obtained from all participants in the study (annex II).

Chapter 5: Results

A total of 177 Health Care Workers (HCWs) from Mekelle Hospital (MH) and Ayder Referral Hospital (ARH) were screened for nasal and hand carriage rate of MRSA. The age range of the participants in MH and ARH was 27-44 years (mean=35.4) and 23-44 years (mean=30.5) respectively. The mean number of years in service and the duration of stay in single unit were 14.7 and 9.1 months in MH and 4.7 years and 7.3 months in ARH. The mean age of the study participants was 32.1 years. Majority of the participants screened for MRSA carriage in the study were nurses 123(69.5 %) and doctors 20(11.3%). A total of 71(40.1 %) males and 106(59.9 %) females were participated in the study with a male to female ratio of 1:1.5 (**Table 5.1**).

Table 5.1. Distribution of HCWs at MH and ARH in relation to age, sex, service years, profession and duration of work in single ward from Nov 2010 - Jan 2011.

parameter	**MH** Frequency(n=73) No (%)	**ARH** Frequency (n=104) No_%)	Total No. (%) (n=177)
Age in year			
18-29	11(6.2)	91(87.5%)	102(57.6)
30-39	33(45)	8(7.7)	41(23.2)
40-44	29(39.7)	5(4.8)	34(19.2)
Sex			
Males	19(26)	52(50)	71(40.1)
Females	54(74)	52(50)	106(59.9)
Service years			
2-3	7 (9.6)	40 (38.5)	47(26.6)
4-5	8(11)	47(45.2)	55(30.1)
6-7	7(9.6)	19(18.3)	26(14.7)
>7	51(69.9)	18(17.2)	69(40)

Profession			
Medical Doctors	8(11)	12(11.5)	20(11.3)
Nurses	50(68.5)	73(70.3)	123(69.5)
Lab technicians	6(8.2)	6(5.9)	12(6.8)
Pharmacists	6(8.2)	3(2.8)	9(5.1)
Physiotherapist	3(4.1)	3(2.8)	6(3.4)
X-ray technicians	------	3(2.8)	3(1.7)
Anesthetist	-------	4(3.9)	4(2.3)
DWSW(m)			
0-1	3(4.10)	11(10.6)	14(7.9)
2-6	24(32.9)	29(27.9)	53(30)
7-12	5(6.9)	6(5.8)	11(6.2)
>12	41(56.2)	58(55.7)	99(56)

Key: MH=Mekelle Hospital, ARH= Ayder Referral Hospital, HCW= Health Care Workers
DW=duration of work in single ward (monthly)

The anti microbial sensitivity patterns of the S. *aureus* isolates against oxacillin showed that 36(43.9%) 0f the total *S. aureus* isolates were resistant (**Table 5.2**).

Table 5.2. Oxacillin profile of the 82 *S. aureus* isolates of nasal and hand swabs from HCWs at MH and ARH Nov 2010 - Jan 2011.

Antimicrobial agent	*Staphylococcus aureus* (n=82)			
	Resistant		Sensitive	
	No	**%**	**No**	**%**
Oxacillin	**36**	**43.9**	**46**	**56.1**

Out of the 73 HCWs screened in MH, the proportion of positive males and females for MRSA carriage was 6(8.2 %) and 17(23.3 %), respectively giving a male to female carriage ratio of 1:2.8 ($P<0.05$). From the 104 HCWs in ARH screened for MRSA, the sex distribution of MRSA was 5(4.8 %) and 8(7.9 %) for males and females respectively with a male to female carriage ratio of 1:1.7. ($P>0.05$). The total MRSA carriage rate in ARH and MH was 13(12.5 %) and 23(31.5 %), respectively. High MRSA was isolated from HCWs in MH than ARH ($p=0.039$).

Over all, females 25(14.1 %) were highly colonized by MRSA than males 11(6.2 %) (P = 0.044, Odds ratio = 1.41) (**Table 5.3**). There was no statistically significantly association between MRSA carriage rate and antibiotic use, skin disease, hypertension, working in one ward, history of diabetes and age in this study (P<0.05).

Table 5.3. Distribution of MRSA by sex among HCWs at MH and ARH Nov 2010- Jan 2011

Sex	No. of samples taken			Hospital carriage of MRSA No. (%)		MRSA carriage rate	
	MH	ARH	Total	MH (n=73)	ARH (n=104)	(n=177)	No. (%)
Males	**19**	**52**	**71**	**6(8.2)**	**5(4.8)**	**11(6.2)**	
Females	**54**	**52**	**106**	**17(23.3)**	**8(7.9)**	**25(14.1)**	
Total	**73**	**104**	**177**	**23(31.5)**	**13(12.5)**	**36(20.3)**	

Key: MH=Mekelle Hospital, ARH= Ayder Referral Hospital

Among the 73 participants of MH who gave nasal and hand swabs, there were 48(65.8%) isolates of *S. aureus.* Out of these 23(47.9%) were MRSA with a total hospital carriage rate of (31.5 %). There were 34(32.7 %) *S. aureus* isolates in ARH, of these 13(38.2 %) of them were MRSA with a total hospital carriage rate of (12.5 %). Higher numbers of MRSA were isolated from nasal swabs 25(14.1 %) than hand 11(6.2 %) (P<0.05) indicating that it is indeed a major reservoir of MRSA. Over all, among the 177 HCWs screened, 82 (46.3%) were found to be carriers of *S. aureus*. Of these, 36 were carriers of MRSA (20.3% of all HCWs) (**Table 5.4).**

Table 5.4. Hand and nasal carriage of S. *aureus* and MRSA in HCWs at MH and ARH Nov 2010 - Jan 2011

Sites of sample collection	No. of *S .aureus* isolates			No. of MRSA carriage		Total MRSA carriage rate No. (%) (n=177)
	MH (n=73)	**ARH (n=104)**	**Total (n=177)**	**MH (n=73)**	**ARH (n=104)**	
Nose	**27**	**22**	**49**	**16**	**9**	**25(14.1)**
Hand	**21**	**12**	**33**	**7**	**4**	**11(6.2)**
Total No. (%)	**48(65.8)**	**34(32.7)**	**82(46.3)**	**23(31.5)**	**13(12.5)**	**36(20.3)**

Key: MH=Mekelle Hospital, ARH= Ayder Referral Hospital, MRSA=Methicillin resistant *S. aureus*

MRSA was isolated from all wards in MH except pharmacy and ICU with the highest proportion in Surgical 7(9.6 %), Medical and OR/Recovery 4(5.5 % each). High carriage of MRSA was isolated from OR/Recovery 7(6.6 %) and Surgical 2(11.9 %) in ARH. Overall, high carriage rate of MRSA per wards was seen in OR/Recovery 11(6.2 %), Surgical 9(5.1%) and Medical wards 5(2.8 %), respectively (**Table 5.5**).

Table 5.5.Distribution of MRSA in different wards at MH and ARH Nov 2010- Jan 2011

Wards/Units	MRSA carriage per ward No. (%)		Total MRSA carriage rate (n=177) No. (%)
	MH(n=73)	ARH(n=104)	
Medical	**4(5.5)**	**1(1)**	**5(2.8)**
Gyn/Obs	**2(2.7)**	**-------**	**2(1.1)**
OPD	**2(2.7)**	**1(1)**	**3(1.7)**
ICU	**--**	**1(1)**	**1(0.6)**
Surgical	**7(9.6)**	**2(1.8)**	**9(5.1)**
Pediatric	**2(2.7)**	**-------**	**2(1.1)**
OR/recovery	**4(5.5)**	**7(6.6)**	**11(6.2)**
Laboratory	**1(1.4)**	**-------**	**1(1.1)**
Physiotherapy	**1(1.4)**	**1(1)**	**2(1.1)**
Total MRSA No. (%)	**23(31.5)**	**13(12.5)**	**36(20.3)**

Key: MH=Mekelle Hospital, ARH= Ayder Referral Hospital

The distribution of MRSA across the different profession/specialty revealed that nurses 19(26.03 %) followed by doctors 2(2.7 %) were the most colonized HCWs in MH. Whereas, in ARH nurses 7(6.8%) and anesthetists 3(2.9 %) were more colonized. No MRSA was isolated from pharmacist in both hospitals. The overall result of this study showed that nurses 26(14.7 %) and medical doctors 4(2.3 %) were the most MRSA colonized HCWs (**Table 5.6**).

Table 5.6. Profession related MRSA distribution in HCWs at MH and ARH Nov 2010- Jan 2011

Profession	Hospital carriage rate of MRSA No. (%)		Total MRSA carriage No. (%) (n=177)
	MH(n=73)	ARH (n=104)	
Medical doctors	**2(2.7)**	**2(1.9)**	**4(2.3)**
Nurses	**19(26.03)**	**7(6.8)**	**26(14.7)**
Lab technician	**1(0.1)**	**--------**	**1(0.6)**
Physiotherapist	**1(2.7)**	**1(1)**	**2(1.1)**
Anesthetist	**--------**	**3(2.9)**	**3(1.7)**

Key: MH=Mekelle Hospital, ARH= Ayder Referral Hospital, HCWs= health care workers

Service year of HCWs in our study was associated with high MRSA colonization. There was high rate of MRSA isolates in the hands and nose of HCWs as their service year increases. Those health profession with service year above seven years were more colonized (58.3 %) than those below seven years (P<0.05) (**Table 5.7**).

Table 5.7. Isolation of MRSA Vs service years of HCWs at MH and ARH Nov 2010- Jan 2011.

Service in years	MRSA isolated No. (%)		Total carriage No (%) (n=36)
	MH	ARH	
2-3	**1(14.3)**	**2(5)**	**3(8.3)**
4-5	**4(50)**	**1(2.1)**	**5(13.9)**
6-7	**3(42.9)**	**4(23.5)**	**7(19.4)**
>7	**15(29.4)**	**6(33.3)**	**21(58.3)**
Total	**23**	**13**	**36(100)**

Key: MH=Mekelle hospital, ARH= Ayder referral hospital, HCWS= Health care workers

The drug susceptibility pattern of the (n= 36) MRSA isolates to other nine commonly used antibiotics done by Kirby-Bauer disk diffusion method indicated that they were multidrug resistant. High level of resistance (>80%) in MH was seen for Tetracycline and Ampicilin (nose =87.5 %, hand =100 % each), Amoxicillin (nose = 81.3 %). Intermediate level of resistance (60-80%) for Amoxicillin (hand = 71%), Chloramphenicol (nose = 62.5%, hand = 71.4 %) and (nose =62.5 %). Low level of resistance (<60 %) in this hospital was seen for Gentamycin (nose = 37.5 %), Ceftriaxone (hand = 42.9 %). Erythromycin (nose = 43.8%, hand = 57 %) and Vancomycin (5.6 %). Only two MRSA form nose was observed resistant for Vancomycin which were also resistant to all antibiotics tested.

Among the MRSA isolates in ARH high level of resistance (>80 %) was seen for Ampicilin (nose = 100 %, hand = 100 %) and intermediate level of resistance (60-80 %) for Gentamycin (hand71.4%), Tetracycline (nose =77.8 %, hand =75 %), Chloramphenicol and Amoxicillin (nose =66.7 % each). Low resistance was seen for Gentamycin (nose = 22.2 %, hand = 25 %), Erythromycin and Amoxicillin-Clavulanic Acid (nose =33.3 % each, hand = 25 % and 50 % resp.), Ceftriaxone (nose = 44.4 %, hand = 55.6 %) and none of the isolates of MRSA from ARH were resistant for Vancomycin.

The overall susceptibility patterns of MRSA isolates against nine antimicrobial agents showed high level of resistant (>80 %) for Ampicilin and Tetracycline, intermediate resistance (60-80 %) for Amoxicillin and Chloramphenicol and low resistance (<60 %) was seen for Amoxicillin-Clavulinic Acid, Ceftriaxone, Erythromycin, Gentamycin and Vancomycin (**Table 5.8**).

Table 5.8. Susceptibility pattern of the 36 MRSA isolates to other antibiotics at MH and ARH Nov 2010 - Jan 2011

Antimicrobial agents	MRSA isolated in MH		MRSA isolated in ARH		Total resistance rate (n=36) No. %)
	Nose (n=16) Resistance No. (%)	Hand (n=7) Resistance No. (%)	Nose (n=9) Resistance No. (%)	Hand (n=4) Resistance No. (%)	
ACA	**9(56.3)**	**2(28.6)**	**3(33.3)**	**2(50)**	**16(44.4)**
Ceftriaxone	**10(62.5)**	**3(42.9)**	**4(44.4)**	**2(50)**	**19(52.8)**
Chloramphenicol	**10(62.5)**	**5(71.4)**	**6(66.7)**	**3(75)**	**24(66.7)**
Erythromycin	**7(43.8)**	**4(57.1)**	**3(33.3)**	**1(25)**	**15(41.7)**
Gentamycin	**6(37.5)**	**5(71.4)**	**2(22.2)**	**1(25)**	**14(38.9)**
Ampicilin	**14(87.5)**	**7(100)**	**7(77.8)**	**4(100)**	**32(88.9)**
Tetracycline	**14(87.5)**	**7(100)**	**7(77.8)**	**3(75)**	**31(86.1)**
Vancomycin	**2(5.6)**	**----------**	**---------**	**--------**	**2(5.6)**
Amoxicillin	**13(81.3)**	**5(71.4)**	**6(66.7)**	**3(75)**	**27(75)**

Key: ACA =Amoxicillin-Clavulanic Acid; MH= Mekelle hospital, ARH= Ayder referral hospital

Chapter 6. Discussion

Methicillin resistant *Staphylococcus aureus* (MRSA) is the most significant pathogen responsible for hospital and community based infections. The spectrum of MRSA infection varies from mild skin infection to serious and invasive disease such as septicemia, pneumonia, endocarditis, deep seated abscess, food poisoning and toxic shock syndrome (TSS) which are associated with worse outcome in addition to prolonged hospital stay and higher cost of treatment. MRSA strains are resistant to a group of antibiotics for which reason the treatment of infections due to this organism is always challenged especially when critically ill patients are infected (Makgotlho, 2009).

The role of MRSA carriers in the transmission of this pathogen is serious. They transmit the organism to another person via direct contact, usually through colonized hands and aerosolization following sneezing. Therefore, health care workers who are at the boundary between the hospital and the community may serve as agents of cross-transmission of HA-MRSA and CA-MRSA. Likewise, screening and eradication of MRSA from colonized health workers is an important part of a comprehensive infection control policy for the bacteria (Azeez-Akande, 2010).

Our result of nasal and hand swabs collected from 177 participants during the period of Nov/2010 - Jan /2011 showed significant number the HCWs 82(46.3 %) were *S. aureus* carriers in their anterior nares and hands. MRSA was isolated from 36(20.3%) of the HCWs. 23(31.5 %) of them were from Mekelle hospital and the rest 13(12.5%) were from ARH.

High MRSA carriage rate was observed in MH than ARH. This could be due to the difference in length of services the HCWs and the hospitals provided to the community. MH was giving service since the last 50 years and the HCWs in this hospital have worked longer time than in the newly established ARH that started service since 2006. So, this long service might contribute to the high chance of the HCWs in MH to be colonized by MRSA (Albrich and Harbarth, 2008). Poor hygienic practice and infection control in MH might also be the possible reasons for the high MRSA isolation.

MRSA carriage rate (20.3 %) of HCWs in this study was in agreement with the results from Ethiopia (Gabriel and Kebede, 2007), Cairo (22 %) (Abdel Rhaman, 2010) and Nepal (23 %) (Mihreta *et al,* 2010). However, it was lower than a study conducted nasal and hand MRSA carriage rate in critical units in Nigeria (52.5 %) (Aderibigbe *et al,* 2010), in Pakistan (29 %) (Farzana *et al,* 2008), in India (50 %) (Aravind *et al,* 1999) and (93 %) (Albrich and Harbarth, 2008). This might be due to the fact that the study participants in Nigerian and Indian were from especial units ICU and burning units. In addition to this, MRSA identification in Nigeria was done by an overnight incubation of the swabs before inoculating in MSA that could have increased the rate of MRSA isolation.

On the other hand, our findings was higher than the study results in nasal carriage rate of MRSA studies conducted in Iran (5.3%) (Askariana *et al,* 2009), Turkey (14.3%) (Yasemin *et al*, 2009), USA (6%) (David *et al*, 2008), Taiwan (3%) (Poliagle *et al*, 2007), Auckland University of Technology (0 %) (Fadheel *et al, 2008).* This higher result might be due to the number of swabs which we have collected both from nasal and hand swabs where as these studies were done only from nasal swabs. Furthermore, our result was higher than the surveillance report in nasal MRSA in HCWs in Africa (15%), E. Asia (13%), Australia (9.5%) and N. America (4.25%) (Albrich and Harbarth, 2008). The difference in design of the studies, such as sample size and study subjects, geographical difference, method of MRSA identification, hospital set up and difference in the policy of infection prevention and control may account for the disparity in the carriage rate of MRSA.

Total MRSA carriage in this study was particularly high among nurses (14.7%**)** and medical doctors (2.3%). This was similar with the results from Nigeria (Aderibigbe *et al,* 2010) and Nepal (Askariana *et al,* 2009) in India (Aravind *et al,* 1999). This high carriage might be due to their long stay with patients. So, this could be seen as a great challenge as doctors and nurses have high frequency of contact with the patients, that enables the bacteria to disseminate to critically-ill patients, their family and ultimately to the community.

High proportion of MRSA isolates in our study were seen in OR/Recovery 11(6.2%), Surgical 9(5.1 %) and Medical wards 5(2.8%), respectively. The high prevalence in OR/Recovery ward could be due to the fact that the patients admitted to this units stay in the recovery ward and they may acquire MRSA from the environment and nearby patients through their body part that has under gone operation and may easily transmit to the HCWs when they take care of them. The long hospital stay of patients in surgical and medical wards as compared to other wards may contribute for the increasing chance of MRSA colonization (Gabriel and Kebede, 2007; Mihreta *et al*, 2010).

The distribution of MRSA carriage by sex indicated that females 25(14.1%) were more colonized than males 11(6.2%) (P = 0.044, Odds ratio = 1.41) which is contradictory with studies done in Uganda (Ajay *et al*, 2010), Turkey (Balc *et al*, 2009) and South India (Mathanra *et al*, 2009*)* that revealed high MRSA carriage rate in males than females. This might need further study to know the exactly reason behind the high rate of colonization of females than males or vice versa.

Service year of HCWS in our study was associated with high MRSA colonization. There was an increasing number of MRSA carriage rate as the HCWs increases their service especially, those HCWs whose service year was above seven years were more colonized (58.3 %) than those with below seven years (P<0.05). This was similar with the study done elsewhere (Balc *et al*, 2009; Albrich and Harbarth, 2008). This high rate of MRSA with long service year could be due to the fact that long duration of service in hospital increases contact of HCWs with patients; hence, high chance for MRSA colonization.

The proportion of nasal carriage 25(15.2%) was higher than hand carriage 11(5.1%) (P<0.05) This was similar with the other studies done elsewhere (Abdel Rhaman, 2010; Aderibigbe *et al*, 2010; Aravind *et al*, 1999). Though the hand has more frequent contact with patients, the low MRSA carriage in the hands of health care workers in our study may be due to the frequent hand washing with different detergents and hence less chance for the bacteria to in habitat in the finger webs. While the nose is with less chance to get the bacteria from the patients than the hand, once the bacteria gets an access to the nose during patient sneezing, coughing or by self contact with un washed hand after contacting patients with MRSA infection it remains there and multiples independently by attaching to the epithelial cell (Belkum *et al*, 2005). This also

might be due to the fact that there is no practice of mupirocin for nasal decolonization among HCWs.

Our study also provides insights into the susceptibility profile of the isolates. From the total 82 nasals and hand *S. aureus* isolates (43.9%) were MRSA and the rest (56.1%) were MSSA. Similar with the study conducted by (Gabriel and Kebede, 2007), The results in anti microbial susceptibility pattern of the MRSA isolates to other commonly prescribed drugs done using the disk diffusion method indicated that it has developed multidrug resistance (resistance for two or more drugs). When the two hospitals are compared to each other, more multidrug resistant of MRSA were seen in MH than ARH. This might be due to the long time service of MH that perhaps lets the chance of existing more resistant species in the hospital environments and health professions than the new ARH.

The susceptibility patterns of MRSA isolated from nasal and hand of the health care workers against other nine commonly prescribed antimicrobial agents showed that high level of resistant (>80%) was seen for Ampicilin and Tetracycline, intermediate level of resistance (60-80 %) for Amoxicillin and Chloramphenicol and low level of resistance (<60 %) for Amoxicillin-Clavulinic Acid, Ceftriaxone, Erythromycin, Gentamycin and Vancomycin.

However, less resistance was seen for Erythromycin (41.7%), Amoxicillin-Clavulinic Acid (44.4 %) than (Aderibigbe *et al*, 2010; Shrestha *et al* 2009) and Gentamycin (38.9 %) (Alborzi *et al*, 2009; Aderibigbe *et al*, 2010; Shrestha *et al*, 2009) were seen in this study. The resistance of MRSA isolates for Gentamycin, Tetracycline and Erythromycin in this result was higher than a study in Nepal national medical college teaching hospital but lower for Ampicilin (Shakya *et al*, 2010). This difference in susceptibility profile of MRSA for other antibiotics might be due to the difference in usage of these agents for treatment of patients in different geographic locations.

Only 2(5.6%) MRSA isolates from nasal swabs were found to be Vancomycin resistant which were also resistant for all the nine antibiotics we used in this study. This low level of Vancomycin resistant isolates in the hospitals indicates that in an event of outbreak of hospital MRSA infection, Vancomycin could be effective. However, the spread of the only Vancomycin resistant isolates could be a challenge since they were resistant for all the antibiotics tested in the study. Thus, the anti biotic profile of the bacteria in our study indicated that MRSA has developed multidrug resistance which needs due attention in selection of antibiotics for patient treatment suspecting of *S. aureus* infection.

7. Limitations of the Study

It was impossible to classify whether the HCWs were persistent or transient carriers of MRSA.

The MRSA isolates recovered in this study were not molecularly typed making it difficult to establish the possibility of sharing the same strain among HCWs in the wards.

8. Conclusion and Recommendation

In conclusion, MRSA carriage among HCWs in this study was high. The carriage rate was worse among nurses and doctors though significant number of other HCWs were also carriers. So, this study gives more emphasizes the need for a regular surveillance of MRSA among HCWs to prevent transmission among HCWs, patients, their family and ultimately to the community. The study also revealed that the MRSA has developed multidrug resistance for other commonly prescribed antibiotics which will be amore challenge for treatment of critically-ill patients. Based on these findings the following recommendations are made:

1. Nasal and hand carriage of MRSA in HCWs in this study disclosed high prevalence. So, regular surveillance of HCW for MRSA carriage has to be done.
2. HCWs should apply the infection preventive procedures to decrease the MRSA transmission such as: Hand washing and disinfection after and before patient treatment, cleaning and decontamination of the medical equipments and the environment, proper usage of glove, gown, and masks during patient treatment.
3. The MRSA isolates in this study has developed multi drug resistant a challenge to treat a patients infected by this bacteria. It is recommended that treatment of MRSA infections should be based on culture and sensitivity. Therefore, the diagnostic capabilities of the microbiology laboratory should be strengthened.
4. Long service year and female sex were found as a risk factor for colonization of HCWs by MRSA. Thus, due attention has to be given in screening this group.
5. This study has to be done in the porters, patients and the food handlers, cleaners who have more exposure to in the hospital wards using the recent techniques like molecular methods (PCR) to know the prevalence of the bacteria.
6. The prevalence of MRSA in this study was high. Therefore, more attention needs to be given to this problem in Ethiopia to define its magnitude, risk factors and associated complications.

9. References

Abdel Rhaman. (2010) Antimicrobial resistant bacteria among HCWs in ICU at Ain sham University Hospitals. *J Egypt socxparasitol* **40**(1):71-83.

Aderibigbe A, Adeboye MA, Adesiyun O, Bolaji BO, Desalu O , Fadeyi A Fowotade A, Nwabuisi C, Oyedepo OO, Olanrewaju T.O, Salami A.K.(2010) Methicilin Resistant *Staphylococcus aureus* Carriage amongst Healthcare Workers of the Critical Care Units in a Nigerian Hospital. *American Journal of Infectious Diseases* **6** (1): 18-23.

Ajay KS, Chang S, Achilles K, Kirenga B, Bwanga F, Joloba M, Donskey C. (2010)Prevalence of nasal carriage of MRSA in patients and healthcare workers at Mulago Hospital in Kampala, Uganda. International conference on health care –Associated infections http://shea.confex.com/shea

Albrich WC, Harbarth S. (2008) Health-care workers: source, vector, or victim of MRSA. *Lancet Infect Dis* **8**(5):289-301.

Alberta P, Louie F. (2008) Epidemiology of MRSA, Differences between CA and HA MRSA Accessed in http://www.chica.org/bd

AlborzicA, AskarianaM, JaponibA, Zeinalzadeha A. (2009) Prevalence of Nasal carriage rate of MRSA and its anti biotic susceptibility pattern in HCWs at Namazi Hospital, Shiraz, Iran. *International J.inf.Dis* **13** (5):241-247

Ali Khan JA, Akhtar NA, Farzana K, Nasir B, Rashid Z, Sattar A.(2008) Nasal Carriage of Staphylococci in Health Care Workers: antimicrobial Susceptibility Profile. *Pak. J. Pharm. Sci* **21** (3):290-294.

Aravind P, Krishana PU, Srinivasa H. (1999) Screening of burn units staff of tertiary care hospital for MRSA colonization.*MJM 5(2)*:80-84.

Aroutcheva A, Rice T, Hota B, Kyle J, Robert A. Weinstein (2010) Community-Associated Methicilin-Resistant Staphylococcus aureus and HIV .*Clinical Infectious Diseases* **50**:979-987

Azeez-Akande O. (2010) Global trend of Methicilin-Resistant Staphylococcus Aureus and emerging challenges for control. *Afr. J. CLN. EXPER. MICROBIOL **11**(3):150-158*

Balc I, Karagoz DI, Kilic IH, Namıduru M, Ozaslan M, Suner A, Zer Y. (2009) Investigation of nasal colonization of health care workers by methicillin-resistant *Staphylococcus aureus* with using new generation real-time PCR assay: *African Journal of Biotechnology* **8** (20):5542-5546 *accessed in http://www.academicjournals*

Belkum VA, Leeuwen VW, Margreet CV, Melles CD, Nouwen LJ, Verbrugh AH, Wertheim HF.*(2005)* The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* **5**: 751–62

Braunwald, Fauci, Kasper, Hauser, Jameson L, Loscalz O. (2008) Principles of internal medicine. *McGraw-Hill companies, Inc. ed*. 17th. Pp. 814-815.

Belghiti J, Durand F, Fantin B, Frédéric B, Mentré F, Le Mée J, Olivier J G, Zechovsky NL, Zarrouk V.(2000) Association between Nasal Carriage of *Staphylococcus Aureus* and Infection in Liver Transplant Recipients. *Clin Infect Dis* **31** (5): 1295-1299.

Chang FY, Chen YW, Chiu YW, Hsiao CF, Siu LK, Tsai JC.(2010) Methicilin- Resistant *Staphylococcus aureus* Carriage, Infection and Transmission in Dialysis Patients, Healthcare Workers and their Family Members. *J Clin Microbiol Infect Dis **25:** 32- 330*

Cheesbrough M. (2002) District Laboratory Practice in Tropical countries. Vol II. Cambridge University press: England Pp. 225-248.

David B, Mehmel LA, Parenteaus S. (2008) MRSA transmission, the possible importance un recognized HCWs carriage. *AmJ infection control* **36**(2):93-97.

Fadheel ZH, Henderson RA,Perry HE.(2008) Comparison of Methicilin-resistant *Staphylococcus aureus* (MRSA) carriage rate in the general population with the health-worker population. *N Z J Med Lab Sci* **62**:4-6

Gabriel R and Kebede E. (2007) Nasal carriage and drug sensitivity of *Staphylococcus aureus* among health workers of Jimma University specialized hospital, southwestern Ethiopia. *Ethiop J Health Sci* **17**(2).

Gustavo PK, Marines DV, Igor MM.(2001) High frequency of colonization and absence of identifiable risk factors for MRSA in intensive care unit in Brazil. *Braz J. infect. Dis* **5**(1):1-7.

Kluytmans J, Struelens M**.** (2009) Methicillin resistant *Staphylococcus aureus* in the hospital. *BMJ;338:doi:10.1136/bmj.b364.*

MakgotlhoP.(2009) Molecular characterization of MRSA strains,

Accessed in (http://upetd.up.ac.za/thesis/available/etd02182010172959/unrestricted/dissertation

Mathanra J, Sujath A, Sivasangeetha SC, Parij A. (*2009)* Screening For Methicilin-Resistant *Staphylococcus Aureus* Carriers among Patients and Health Care Workers of a Tertiary Care Hospital in South India. *Indian Journal of Medical Microbiology **27(1): 62-4.***

Matouskova I, Janout V. (2008) Current knowledge of Methicillin-resistant staphylococcus *Biomed Pap M 152*(2):191–202.

Mihreta T, Shakya B, Shresth S. (2010) Nasal carriage of MRSA among a national Medical College Teaching Hospital, Birgunj, Nepal. *Nepal med coll* **12**(1):26-29.

Mohaparta TM, Pokhrel B, Shrestha B. (2009) *S.aureus* nasal carriage among HCWs in Nepal hospital. *Braz J infect Dis* **13** (5).

Sampathkumar P. (2007) Methicillin-Resistant *Staphylococcus aureus*: The Latest Health Scare. *Mayo Clinic Proceedings* **82** (12): 1463-1467.

Wayne PA. (2002) Performance standard of anti microbial susceptibility NCCLS approved standard M, National Committee for Clinical Laboratory Standards.

10. Annexes

10. 1.Annexes I Questioner

Questioners for investigation of nasal and hand carriage rate of MRSA and its pattern of antimicrobial resistance in Ayder Referral Hospital and Mekelle Hospital, Mekelle, Tigray, Ethiopia.

I Demographic and history of MRSA infection

Serial number ----------

1. Age

   - ☐ 18-29
   - ☐ 30-39
   - ☐ 40-44
   - ☐ >44

2. Sex

   - ☐ Males
   - ☐ Females

   - ☐ Number of years in health service
   - ☐ 2-3
   - ☐ 4-5
   - ☐ 6-7
   - ☐ >7

3. Wards / unit

   - ☐ Surgical
   - ☐ Medical
   - ☐ Pediatrics
   - ☐ Gynecology
   - ☐ ICU,
   - ☐ pharmacy
   - ☐ OPD
   - ☐ X-ray
   - ☐ Laboratory
   - ☐ Physiotherapy

4. work duration in one ward (in month)

   - ☐ 0-1
   - ☐ 2-6
   - ☐ 7-12
   - ☐ >12

5. Category of work / speciality

   - ☐ Doctors
   - ☐ Nurses
   - ☐ Laboratory tech
   - ☐ Radiologist
   - ☐ Physiotherapist
   - ☐ Pharmacist

6. History of diabetic

☐ Yes

☐ No

7. History of hypertension

☐ Yes

☐ No

8. History of currently skin disease

☐ Yes

☐ No

9. History of previous antibiotic treatment

☐ Yes

☐ No

If yes which drug did you take? ---

Date and time specimen collection -----

II Laboratory data

1. Type of specimen - swab
2. Site of specimen nose and hand
3. Organism isolated from nose CPS/ CNS/None
4. Organism isolated from hand CPS/ CNS/None

5. Anti microbial susceptibility test	S (mm)	I (mm)	R (mm)
Amoxy clavulanic acid	------	-- ---	------
Gentamycin	--------	-------	-------
Chloranphenicol	-------	------	-----
Oxacillin/Methicillin	-------	-----	- ---
Tetracycline	-------	------	------
Vancomycin	--------	-------	----
Ceftriaxone	--------	------	-----
Erythromycin,	---------	-----	-----
Amoxicillin	---------	-----	-----
Ampicilin	--------	-------	-------

Name of the principal investigator ----------

Signature ---------- date ----------

10.2. Annex-II Information sheet

Purpose: MRSA infections are major problems in human. It is associated with adverse outcomes of in addition to long stay and loss of economic. The aim of this study is to determine the nasal hand carriage rate of MRSA in HCWs.

Procedure: To determine the prevalence and associated factors of MRSA, we invite you to take part in this study. If you are willing to participate in this project, you will be examined for your MRSA carriage status. We will collect nasal and hand webs swabs.

Risk and Discomfort: As the procedure will be carried out by experienced health professionals with a standard aseptic condition the risk and discomfort are almost none.

Benefits: If you participate in this research, you will get a clinical assessment of your health condition about the MRSA.

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. Which number belongs to which name will be kept under lock and key, and it will not be revealed to anyone.

Right to refuse or withdraw: You have full right to refuse from participating in this research if you do not wish to do so.

Whom to contact:

If you have any further question and in case of urgency you can contact:-

Tel: - 0913504787 E-mail:- araya13e25@gmail.com

10.3. Annex. III. Consent form

Consent form (to be translated to local language)

I have been informed verbally and in writing about this study that plans to investigate nasal and hand carriage rate of MRSA among HCWs. I understand what is involved and I have been requested to give nasal and hand l swab sample for laboratory investigation. I also know whom to contact if I need more information. I understand that confidentiality will be preserved. Moreover, I also understand that I have a right to withdraw from participating in this study at any time and my actions will have no impact on the overall management of my conditions.

The investigator has briefed me there are no risks associated with the swab sample collection.

I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my consent and cooperate at my will to participate fully in the study.

My signature below indicates that I agree to participate in this study.

Subject's signature date of signature

_____-

Signature of Person Obtaining Consent date of signature

Signature of witness

1----------

2----------

Declaration

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

MSc candidate: **Araya G/Eyesus Wasihun**

Signature _____

Date and palace of submission _____

Supervisor: **Solomon G/Slassie, MD, MSc**

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

Supervisor: **Adane mihret, VDM, M.Sc, PhD candidate**

Signature _____

Date and Place of Submission _____

Addis Ababa, Ethiopia