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Nasal Carriage Rate, Antimicrobial Susceptibility Pattern of *S.aurues and* Methicillin Resistant *S.aurues* among Health Care Workers and their Knowledge, Attitude and Practice regarding this organism at Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Lensa Makulo, entitled:
Nasal Carriage Rate and Antimicrobial Susceptibility Pattern of *S.aurues* among Health Care Workers and their Knowledge, Attitude and Practice regarding Methicillin Resistant *S.aurues* at Zewditu Memorial Hospital, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

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Abbreviations

AAU	Addis Ababa University
AHB	Addis Ababa Health Bureau
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
BAP	Blood agar plate
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CLSI	Clinical and laboratory Standards Institute
DRERC	Departmental Research and Ethics Review Committee
HCWS	Health Care Workers
KAP	Knowledge, Attitude and Practice
MSA	Mannitol Salt Agar
MHA	Muller Hinton Agar
MRSA	Methicillin Resistant *S.aurues*
MSSA	Methicillin sensitive *S.aurues*
PI	Principal Investigator
SOP	Standard Operating Procedure
SPSS	Statistical package for Social Sciences
WHO	World Health Organization
ZMH	Zewditu Memorial Hospital

Abstract

Background: *S.aurues* is gram-positive coccus which is a normal flora of the skin and nose of the healthy population. However it can cause different types of infections ranging from minor skin infections to life threatening conditions such as septicemia, pneumonia, endocarditis, meningitis and osteomyelitis.

Objective: The objective of this study was to determine the nasal carriage rate and antibiotic susceptibility profile of *S.aurues* and methicillin resistant *S.aureus* among health care workers and their knowledge, attitude and practice regarding methicillin resistant *S.aureus* at Zewditu MemorialHospital.

Methods: A cross-sectional study was conducted on178 HCWs at Zewditu memorial hospital in city of Addis Ababa, Ethiopia from February to May 2021 on isolation of nasal *S.aurues* and determination of antimicrobial resistance profile. Standard microbiological methods were used to isolate and identify *S.aurues* from nasal swabs obtained from study subjects. Susceptibility profiles to different antibiotics was done using Kirby-Bauer disk diffusion method. Data on socio-demographic factors and knowledge, attitude and practice related data were collected using well-structured questionnaires. The collected data was organized and tabulated using SPSS software (version26).

Results: Prevalence of *S. aureus* and MRSA in this study were 12.9% (23/178) and 7.9% (14/178) respectively. Rate of MRSA among *S. aureus* was 60.9 % (14/23). In our study, MRSA carriage was higher among nurse professionals (11.1%) and surgical ward (28.6%). None of the MRSA isolates were sensitive to penicillin. However, low resistance was observed for chloramphenicol and clindamycin. Among 178 participants, 150 (84.3%) were within adequate knowledge range while, 28(15.7%) of the participants had showed poor knowledge about MRSA. Concerning practice of HCWs; only half (97(54.5%)) of them had good practice on prevention of MRSA whereas; 81(45.5%) of the participants had poor practice.

Conclusion: In this study, the overall prevalence of MRSA was 7.9%. Clindamycin and chloramphenicol were preferable antimicrobials for treatment of MRSA and *S. aureus*. This study had also showed limited KAP of HCWs regarding MRSA prevention; therefore, continuous and effective education should be provided for HCWs.

Keywords:- *S.aureus*, Drug Resistance, Health care worker, Ethiopia

1. Introduction

1.1 Background

S. aurues is gram positive, non-motile, non-spore former and facultative anaerobe having the ability to produces coagulase and DNAse and referred to as coagulase positive *staphylococcus.* It appears as grape like clusters under microscope and ferments mannitol producing golden yellow colony on mannitol salt agar (a selective and differential medium for *S. aurues*) and produce beta hemolysis on sheep blood agar medium (1-4).

This bacteria act as a commensal bacterium, asymptomatically colonizing the anterior nares of 20% to 30% of the human population which serves as reservoir for the spread of the pathogen to other parts of the body.However it is an important opportunistic human pathogen and frequent cause of both nosocomial and community acquired infections associated with high mortality and morbidity around the world.Nasal carriage has been shown to play a key role in the pathogenesis of *S. aurues* infections in patients undergoing surgery, dialysis, and in intensive care unit (ICU) with higher infection risks in persistent carriers(2,5-7).

The factors such as low level of immunity among hospitalized patients especially in HIV and diabetes mellitus, high number of invasive examinations and treatment, poor patient care practices and hospital environment may contribute to spread of infections among patients (2, 3).
The organism can establish solid interactions with nasal epithelial cells via various proteins and many cell surface components, thus transforming into persistent carriage. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins and the expression of a cell surface protein that binds and inactivates antibodies (2,8).It is the most dangerous of all of the common staphylococcal bacteria and it spread by having direct contact with an infected person, contaminated object, contaminated food and rarely by inhaling infected droplets dispersed by sneezing or coughing especially in hospitals and other health institutions(7).

Resistance to antibiotics is associated with an increase in disease severity, which will increase length of hospitalization, mortality, treatment costs and need for use of alternative drugs. MRSA is a strain of *S. aureus* which is resistant to beta-lactam antibiotics resulting into treatment challenge (7, 9).

HCWs play a significant role in MRSA transmission at health care institutions. Colonised HCWs have been implicated as a source of infection for their patients and themselves as they propagate it to different site of infection through their hands. Knowledge, attitude and practice acquired by HCWs has a vital role in prevention this infection. Therefore assessment of their KAP level may contribute in generation of an environment in which infection of MRSA is of less problem. (10,11, 12).

1.2 Statement of the Problem

The global emergence of multidrug resistant *S.aurues* is a growing threat to antibiotic therapy. Healthcare-associated methicillin-resistant *S.aurues* is associated with significant mortality& morbidity and imposes a serious economic burden on healthcare resources worldwide(2,9,14, 15)

European Antimicrobial Resistance Surveillance System reported that prevalence of HA-MRSA ranges between 1% and 24%.In United States, although the hospital blood stream MRSA infection rate dropped significantly (70%) from 2005-2012; since 2012, the rate of decline has slowed (17.1%)(15,16).

Although the frequency and impact of MRSA is well documented in western countries far less is known about the incidence and burden of this infection in non-industrialized and newly industrialized regions of the world including Ethiopia. Studies conducted in different countries of Africa indicate that prevalence of MRSA within country and across country is heterogeneous with prevalence of 34.6% reported in Cameroon, 3.7% in Kenya, 31.5% in Uganda and 9% in Eritrea (13,15,17).

In Ethiopia, most of antimicrobials are prescribed without antimicrobial susceptibility testing which is one of the major causes for the rapid emergence of multidrug resistance including MRSA resulting colonization of HCWs and patients by multidrug resistant strains. According to study conducted in different Ethiopian regions the rate of isolation of MRSA from HCWs had showed higher variation. In addition, there is a continuous report that shows an increase in the prevalence of MRSA indicating the pathogen has been spreading in very high rate in Ethiopian hospitals (11,13, 18-20).

Even though several studies had showed high colonization rate in addition to low level of KAP regarding MRSA among both the medical staff and community, there is neither surveillance system nor control policy for MRSA in Ethiopia. (11,13).

Since colonization rate, resistance pattern and awareness of HCWs about MRSA varies from place to place; this study was aimed to determine the nasal carriage rate, antibiotic resistance profile and KAP of HCWs regarding MRSA at Zewditu Memorial Hospital.

1.3. Significance of the study

The result of this study had provided the actual current situation of *S.aureus* and MRSA nasal carriage among HCWs which may give overview of the degree of disease transmission at hospital setting, indicating importance of periodic Surveillance.

The study had also determined the AST pattern of this organism which might be act as an early warning signal of potentially pathogenic strain.

In addition the study had also assessed HCWs awareness, perception and practice regarding MRSA which could identify gap of KAP pattern that may identify needs, problems and barriers.

Therefore, this evidence based information would give additional input for program planners and policy makers working in the area of infectious disease.

Finally, the results of this study could be used as reference for further similar studies that will be conducted in Addis Ababa, Ethiopia in the future.

2. Literature review

2.1. Nasal carriage rate and AST pattern Of *S. aurues* among HCWs

In systematic review of articles published between January 2000 and December 2013 on MRSA carriage among HCWs in non-outbreak settings in Europe and United States; 31 studies were included in this review. The pooled MRSA colonization rate was 1.8% (95% CI). The pooled MRSA nasal carriage was highest in nurse professionals (6.9%). The meta-analysis of all studies had reported MRSA colonization rates for nurses had showed increased risk of colonization (2.58 times higher than that of other HCWs); even though, the $\chi 2$ test showed no evidence of heterogeneity (21).

In study conducted at Tertiary care hospital in Lahore in 2017; of the 230 nasal samples taken from HCWs 14.3% were positive for *S. aurues*. Of these 36.3% were MRSA positive. More than 30% of doctors and nurses were carriers of *S.aurues* while the colonization rates in Operation Theatre Assistants was lower at 12.1 % (22)

Another cross sectional study conducted in Nikuee Hospital, Qom, Iran, in 2012; 152 health care workers (HCWs) were assessed to determine the frequency of nasal carriage of *S. aurues* and their antibiotic resistance profiles and 34.2% illustrated nasal carriage of *S. aurues*, and among them, 23.5% were carriers of MRSA. None of the isolates were resistant to vancomycin. However, 96.1%, 33.3%, 27.5%, 19.6%, 9.8%, and 15.7% resistance to penicillin, erythromycin, clindamycin, cefazolin, co-trimoxazole, and doxycycline were observed (5).

In study conducted at Nepal in 2013; of 204 HCWs, 32(15.7 %) were nasal carriers of *S. aurues* and among them 7(21.9 %) were carrier of MRSA. The overall nasal carriage rate of MRSA was 3.4 % (7/204). *S. aurues* carriage rate was highest among doctors 20.8 % (15/72) while MRSA carriage rate was highest among nurses 7.8 % (4/51).Among the MRSA found 28.6 % (2/7) were from HCWs of surgical wards and operating room each. Resistance of isolates to penicillin was 71.9 % and 42.9 % (3/7) were resistant to erythromycin and gentamycin; whereas all of the isolates were sensitive to, amikacin, teicoplanin and vancomycin (23).

According to study conducted at Al Shifa hospital in Gaza Strip in 2015,the rate of colonization of *S.aurues* and MRSA among 200 HCWs were 62 (31%)and 25.5% respectively. The rate of MRSA among *S. aureus* was 51 (82.3%). MRSA colonization rate was higher among nurse

professionals (30.4%) while carriage rate was lower among doctors (16%). Most of MRSA carriers were from internal medicine and surgical ward departments which was 41.3% and 35% respectively. All *S. aurues* isolates were not sensitive to penicillin. The variable degrees of *S.aureus* sensitivity to different antibiotics including gentamicin, ciprofloxacin, rifampicin, clindamycin and vancomycin was 96.7%, 90.3%, 88.7%, 88.7% and 85.4% respectively. However, sensitivity pattern of the MRSA isolates to gentamicin, ciprofloxacin, rifampicin, clindamycin, tetracycline and vancomycin was slightly decreased (92.2%, 88.2%, 88.2%, 86.3%, 86.3% and 84.3%) (24).

Study conducted at Cantonment Military Hospital, Bahawalpur, Pakistan from Jan - July 2016; Out of the total 150 health care workers 33(22%) were MRSA positive. The culture positive isolates revealed that 12 (36%) MRSA were from axillary region while rest 21(64%) from nose (anterior nares). The highest carriage rate for MRSA was amongst the nurses 15(46%) followed by Operation room staff 9(27%), outpatient departments 3(9%) and doctors 3(9%) (2).

In study conducted at tertiary care hospital of South Punjab Pakistan 2017; out of 225 HCWs *S. aurues* was diagnosed in the nasal flora of 24% (54) participants, out of which 9.3% (21) were MRSA. MRSA were diagnosed in 25% lab. technicians, in 10.86% nurses, 6.2% doctors and only 3.2% in paramedics. Regarding antimicrobial sensitivity patterns for MRSA, amikacin and vancomycin were 100% (21) sensitive, clindamycin was 80.9% (17) sensitive and ciprofloxacin was 71.4% (15) sensitive; while oxacillin, Cefoxitin and ceftriaxone was almost 100% (22) resistant. Regarding MSSA, oxacillin, cefoxitin, amikacin, vancomycin and clindamycin were 100% (33) sensitive, ceftriaxone was 75.7% (25) sensitive and ciprofloxacin was 84.8% (28) sensitive (25).

In study conducted at India in 2018; out of 341 subjects, 125 (36.7%) were carriers of *Staphylococcus aurues*. 86 (25.2%) tested positive in both nasal swab and hand swab specimen, 22 (6.5%) tested positive only in hand swab specimen whereas 17 (5%) tested positive only in nasal swab. Only 10 isolates (2.93%) tested positive for MRSA (3).

According to study conducted in Qazvin university hospitals, Iran from October 2016 to April 2017 on *S. aureus* nasal colonization of HCWs; among 198 HCWs, 32 of the participants (16.1%) were carriers of *S. aureus* and overall MRSA carriage rate was identified as 3%.

Most of the carriers (20.3%) were from ICU department. Colonization of nasal *S. aureus* was significantly lower among nurse professionals and educated participants (26).

According to study conducted at Western Australian acute care hospital on MRSA nasal colonization rate of HCWs in 2011; 52/1542 (3.4%) of health care providers were carriers of MRSA in their nasal swab. MRSA carriage rate was higher among nurses (5.2%, $p<0.01$) and Patient Care Assistants (6.8%, $p<0.05$) than among doctors, allied health and other HCWs (27).

In study conducted at Madagascar in 2016; of 1548 nasal swabs 171 (11 %) were positive for *S. aurues*; 20 (1.3 %) of these isolates were identified as MRSA. *S.aurues* was detected in 91of 863 HCWs (10.4 %) and in 80 (11.8 %) of 685 students; however, 14 (1.5 %) HCWs were carriers of MRSA compared to 6 (0.9 %) students. This study had showed low nasal colonization rate of *S. aurues* and MRSA in both population (28).

In study conducted at tertiary referral hospital in Lusaka, Zambia from May to July 2017; *S.aureus and MRSA* carriage rate among HCWs were17.1% (24/140) and 5.7% (8/140). MRSA Colonization rate was variable among different professionals showing higher carriage rate among doctors (7.1%) and nurses (5.8%) (29).

In study conducted at Kampala International University Teaching Hospital, Southwestern Uganda on MRSA nasal colonization among HCWs from September - July 2017, among 97 participants, 28(28.8%) were colonized with *S.aurues* of which 13(46.4%) were phenotypically MRSA(resistant to cefoxitin). The nasal colonization rate and methicillin (cefoxitin) resistance were significantly increased with age ($p<0.01$). Nasal colonization rates across different professions did not show any significant differences. However, laboratory staff (50%) and doctors (30%) were more colonized (30).

According to study conducted in Kenyatta National Hospital on nasal and hand colonization rate among HCWS from February to march 2015; *S. aureus* was isolated from 40% HCWs (25% &15% from nasal and hand respectively).Among those participants 5.6% of individuals had both nasal and hand colonization giving an overall carriage rate of 34.4%.Out of these MRSA carriage rate was 18.9% (31).

In a systematic review and meta-analysis of *S. aurues* on nasal colonization and MRSA in Ethiopia in 2018; ten articles, eight from patients and two from health workers (2495 nasal swabs) were included and enrolled for meta- analysis. Overall estimated pooled rate of nasal colonization of *S. aurues* and MRSA in Ethiopia were 30.90%& 10.94% respectively. Subgroup analysis by study area was conducted, the highest nasal colonization of *S. aurues* was found in studies conducted in Oromia region 47.74%, followed by studies conducted in Amhara region, 25.82% and 10.94% in Tigray region. The magnitude of MRSA colonization rate had also noted as; Oromia region ranked first 21.28%, followed by Amhara region 6.78%, whereas in Tigray region relatively low colonization rate were demonstrated (4.82%) (32)

Across sectional study conducted at Jimma University Medical Center in 2016 reported that among medical students who rotated at least 8 hours per day in the year, the prevalence of *S.aureus* and MRSA was 22.1% (82/371) and 8.4%(31/371) respectively. The rate of MRSA among *S. aurues* was, 37.8% (31/82). MRSA carriage rate was higher among medical intern, 20% (16/80) than clinical year-II 7.2 % (9/125) and year-I 3.6% (6/166) (33).

In study conducted at Mekelle Hospital, North Ethiopia in 2011;out of 177 health care workers, 36 (20.3%) of them were MRSA carriers in their hand and anterior nares. Nurses and medical doctors had MRSA carriage rates of 26 (13.6%) and 4 (2.3%) respectively. The isolated MRSA were resistant to multiple antibiotics; particularly higher resistance to ampicillin (88.9%) and tetracycline (86.1%) was observed. Two (5.6%) of the nasal isolates were vancomycin resistant (19).

In another cross-sectional study conducted at Wukro and Adigrat general hospitals, Tigray, Northern Ethiopia from September to December 2016; out of 242 HCWs 29 (12%)and 14(5.8%) were carriers of *S. aurues* and MRSA respectively. Carriage rate of MRSA among nurses, doctors and midwives professionals were 10 (7.8%), 1 (7.7%), and 2 (6.7%) respectively. The higher rate of colonization with *S. aurues* and MRSA observed in surgical ward were 7 (20.0%) and 6 (17.1%) respectively. The isolates have varying degrees of resistance to different antibiotics; 93.1% of resistance to penicillin followed by kanamycin 65.5%, erythromycin 62.1%, tetracycline 55.2%, cotrimoxazole 51.7%, ampicillin 48.3%, and amikacin 44.8%. Low resistance were found for chloramphenicol 5 (17.2%) and clindamycin 5 (17.2%) (13).

In study conducted at Dessie referral hospital, north Ethiopia in 2011; 28.8% (34/118) of HCWs were colonized with *S.aurues*.15 of the isolates were MRSA giving 44.1% (15/34) among *S.aurues* carriers and overall carriage rate was 12.7% .Among the 57 males tested, 21 (36.8%) and 10 (17.5%) were carriers of *S. aurues* and MRSA respectively. Females were less colonized with *S .aureus* and MRSA; 13/61(21.3%) and 5/61(8.2%) respectively. The number of MRSA carriers was particularly high among nursing staff (21.2%), doctors (12.5%) and technicians (12.5%). No MRSA carriers were isolated among midwives, pharmacists and radiologists professionals (34).

According to the study conducted at the Gondar University Teaching Hospital, from February to May 2012 ; Out of the 302 individuals, 238 (78.8%) were carriers of staphylococci in their nasal nares; of which, 88 (29.1%) were *S. aurues* . Among these isolates 52 (34.4%) were isolated from health care workers and 36 (23.8%) were from supportive staffs. From the 52 isolates of *S. aurues* 7 (13.5%) were MRSA. However, all isolates from non- health care workers were susceptible to methicillin. Among 52 *S. aurues* isolated from health care workers the increased resistance rate was observed to nalidixic acid (82.7%) followed by ampicillin (78.8%), tetracycline (69.2%), penicillin(63.5%) and trimethoprim- sulphamethoxazole (42.3%) whereas lower resistance was observed to kanamycin (26.9%), erythromycin (21.2%) ciprofloxacin (21.2%), methicillin (13.5%), gentamicin (13.5%) chloramphenicol (13.5%) and clindamycin (9.6%)(7).

2.2. Knowledge, Attitude and Practice of HCWs regarding MRSA

In cross-sectional study conducted on 276 of HCWs regarding their KAP towards MRSA at an acute-care hospital in eastern United States in 2012, HCWs strongly agreed that preventive behaviors reduce the spread of MRSA. Majority of them reported that they almost always engage in preventive practices, however observations of hand hygiene had found the lower rates of adherence among nearly all HCWs (35).

According to study conducted in Mississippi on assessment of nurses KAP towards MRSA in 2017; nurses were relatively strong in their general knowledge of MRSA. Most of the participants (93.99%) knew that hand hygiene is effective in preventing the spread of MRSA and 90.91% knew MRSA is transmitted via the hands of healthcare workers. Most of them (93.33%) agreed that gloves should be worn when examining a patient with MRSA.

Almost half of the participants (48.84%) incorrectly believed that alcohol hand gel was not as effective as hand washing in reducing MRSA transmission. 44.44% of them correctly responded that MRSA nasal carriers should be treated with nasal antibiotics (36).

In another cross-sectional study conducted on 300 health care providers from three regional hospitals in Trinidad and Tobago from April to June 2016 regarding their KAP towards infection prevention control; only 20.3% of the participants had good knowledge, 46.7% had positive attitude and 44% had good compliance towards infection prevention and control (37).

In study conducted in Malaysia on 255 nurses from February-june2014; nearly 67.1% of them had a high knowledge level and only 2.4% of the participants had a low knowledge score on MRSA transmission. Approximately 80% of participant had a high perception level and 19.2% had showed moderate perception towards MRSA. Most participants (94.5%) had said that they apply good practice for MRSA prevention and only 5.5% had moderate level of practice (38).

According to study conducted in Korea in 2017; among 139 DHCPs, four (2.9%) were nasal MRSA carriers. Three participants were successfully decolonized with mupirocin but one participant was decolonized with topical fusidic acid after failure to decolonize with mupirocin. Dentists professionals had a higher score on knowledge and practice questions compared with other professionals ($P < 0.05$). There was a significant, positive correlation between attitude and practice scores ($P < 0.01$) (39).

According to study conducted in Palestine on 331 HCWs in 2016; it is clear that participants had poor knowledge level with regard to MRSA in general but had good awareness of MRSA prevention in particular. With regard to standard precautions, HCWs had showed poor adherence to MRSA infection control (40)

In study conducted in Nigeria in 2011, only 103 (52.0%) of the 198 participants were aware of MRSA but all were favorably disposed to screening for the organism. Most HCWs, 124 (87.9%) reported that there were no MRSA control measures in their respective duty post. The age, cadre of work, and number of years in the hospital's critical care units correlated positively with awareness level (41).

A surveillance conducted on assessment of patients and HCW's KAP regarding multidrug resistant organism infections at CHUK, Kigali hospital in Rwanda in 2017 found that 128/250 (51.2%) of health care providers had limited knowledge towards multidrug resistant organisms. 94.4% of HCWs agree with the use of soap and water as being appropriate for hand hygiene; however, only 69.6% of HCWs had reported as they routinely wash their hands after patient examination (42).

In study conducted on knowledge and beliefs towards antimicrobial resistance among Physician and nurses in Amhara region hospitals, majority of the medical staff did not mention the existence of antibiotic-resistant bacteria. Specifically, only 22.3% of physicians and 2.4% of nurses had awareness regarding MRSA. This report indicate that HCWs in hospitals had an information gap on the antimicrobial resistance pattern of their area (43).

Another study conducted on assessment of KAP level of HCWs towards HAIs prevention at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia from January to June 2019; had reported that out of 236 participants, 90% and 57.2% of them had adequate knowledge level and good attitude regarding HAIs prevention, respectively. On the other hand only 36% of the study participants had good compliance. Education status and length of work exposure of the participants were significantly associated with positive attitude and good practice regarding infection prevention ($P < 0.005$) (44).

Similar cross sectional study conducted on Health care giver's KAP towards HAIs control at Dessie referral hospital, Northeast Ethiopia from March 11-17, 2017 had reported that 86.4%, 76.4% and 23% of the participants had adequate awareness, positive perception and good compliance regarding HAIs respectively (45).

An institutional based cross-sectional study conducted on assessment of HCWs awareness and compliance towards HAIs and associated risk factor at DebreMarkos referral hospital, Northwest Ethiopia from May 11–22, 2015 had reported that about two thirds (84.7%) of HCWs had good knowledge level; however only 57.3% of participants had demonstrated a good compliance on infection control. Elders, more work experience and educational levels were significantly associated with both knowledge and compliance of infection prevention (46).

3. Objective

3.1 General Objective:

- To determine nasal carriage rate and antibiotic susceptibility pattern of *S.aurues* among health care workers and their knowledge, attitude and practice regarding methicillin resistant *S. aurues* in Zewditu Memorial Hospital Addis Ababa, Ethiopia from February to May 2021.

3.2 Specific Objectives:

- To determine prevalence of *S.aurues.*
- To determine prevalence of MRSA
- To determine the susceptibility pattern of *S.aurues* and MRSA
- To asses KAP of HCWs regarding MRSA .

4 .Materials and Methods

4.1 Study Area

Zewditu Memorial Hospital established in 1925 E.C around the current Finfinne hall then in 1963 E.C transferred in front of Filwuha, which is located in the Kirkos Sub-City, City Government of Addis Ababa. According to the data obtained from the hospital; now the hospital has 620 health professionals working in different Departments and 274 administrative staff with providing different medical services. This hospital is referral hospital providing comprehensive service about 15,000 inpatients, 160,000 outpatient visits and 11,000 emergency cases per year.

4.2. Study design and period

A cross-sectional study was carried out from February 2021 to May 2021 in Zewditu memorial hospital.

4.3. Population

4.3.1. Source population

All health care workers working at Zewditu memorial hospital, Addis Ababa

4.3.2. Study Population

All healthcare workers who had fulfilled the inclusion criteria

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

All healthcare workers who were volunteer to the study

4.4.2. Exclusion criteria

Participants who had received antibiotics therapy 2 weeks prior to the study and individuals who had nasal bleeding were excluded from the study.

4.5. Study variables

4.5.1. Dependent variables

- *S.aurues* Colonization rate
- Antimicrobial susceptibility pattern of *S.aurues*
- KAP of HCWs regarding MRSA

4.5.2. Independent variables:

- Socio-demographic characteristics
- Age
- Sex
- Profession
- Educational level
- Service year
- Infection prevention training of HCWs

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

Sample size was calculated using the formula for single proportion sample size calculation by taking a 95% confidence level and a 5% margin of error and proportion or prevalence from previous study conducted at Adigrat & Wukro, Mekelle which is 0.12 (12%)and calculated by using the formula,

$$n= (z\alpha/2)^2 p\ (1-p)/d^2$$

Where:

Z=standard normal value corresponding to 95% confidence interval for a two sided test =1.96

P=estimated prevalence of *S.aureus* among HCWs from study conducted in Adigrat &Wukro on nasal carriage rate of *S.aurues* (13) which is estimated to be 12% and

D= margin of error =5%

Then by substituting numbers in to the formula:

n= (1.96)2(0.12) (1-0.12)/ (0.05)2 ~162.

By considering the contingency rate of 10%; n=162+16.2~178.

Therefore 178 samples; those had fulfilled the selection criteria were taken as the sample.

4.6.2 Sampling Method

Systematic sampling technique was used and a total of 178 samples were collected. HCWs were stratified based on their working department and the sample size was allocated for each stratum or department by using proportional allocation sampling formula;

$nj=(n/N)Nj$ where nj _ sample size of j^{th} stratum

Nj – population size of the j^{th}stratum

n _ $n_{1+}n_{2+}n_k$ …….. Total sample size

N _ $N_{1+}\ N_{2\ +}\ N_K$……..Total population size

The first participant was randomly selected and systematic sampling technique was followed to select each study subject depending on the k^{th} interval to recruit the study participants in each stratum by using their list as a sample frame.

K=N/n, Where; N is the number of units in the population and n is the desired sample size

Then by substitution; K=620/178=3.48~3

All of the samples were collected from each department according to this proportion as summarized in table below.

Table1. Sample size allocation for each department

Working department	**Sample size**	**Working department**	**Sample size**
Adult emergency	13	Operation room	18
OPDs	37	Intensive care unit	4
Pediatrics	28	Laboratory	17
Gyne OB	28	Pharmacy	12
Surgical ward	7	Imaging	5
Medical ward	9		
Total			178

Key: Gyne OB, Obstetrics and Gynecology; OPDs, outpatients departments

4.7. Measurement and data collection procedures

4.7.1. Data collection

After obtaining written consent form, data were collected using a well-structured questionnaire designed to obtain socio-demographic data and other relevant information such as age, sex, profession, working Department, service year, history of taking antibiotics and nasal bleeding. In

addition the questionnaire also designed to obtain information including knowledge, attitude and practice of HCWs regarding MRSA to determine the risk of transmission of this infection at hospital setting.

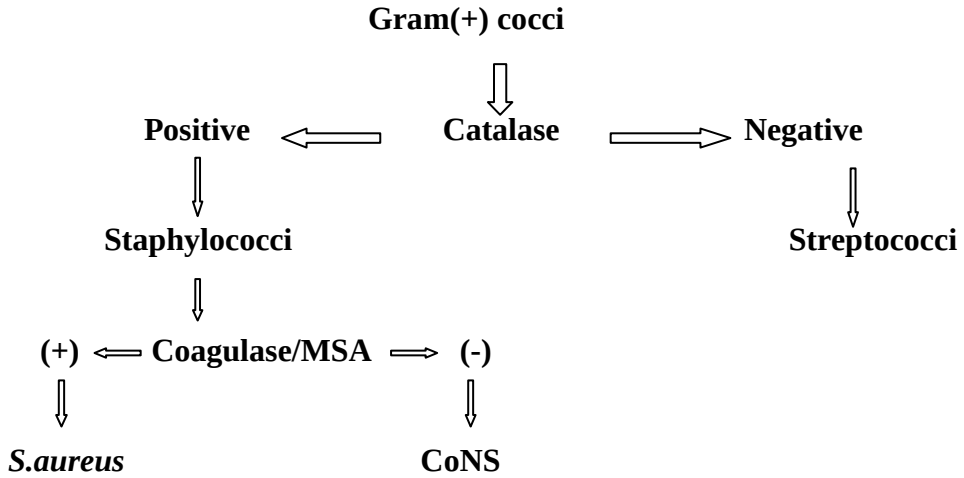
4.7.2 Specimen collection

Sample collection was performed according to the WHO standard for diagnosis of *S. aureus*. A sterile cotton swab moistened with normal saline was passed into the anterior nares of both nostrils of study participants and rotated in both directions to obtain nasal swabs for culture. Swabs were moistened prior to sampling to avoid discomfort. Following collection of specimens each swabs was kept in a sterile test tube and transported to the ZMH Microbiology laboratory within 30 minutes of collection to maintain the viability of the organism (7, 18).

4.7.3 Culture and Identification

Swabs was inoculated on Mannitol Salt agar and incubated at 35-37 °C over night. After 24 hrs incubation it was sub-cultured on sheep blood agar for identification of type of hemolysis. All positive culture was identified by their characteristics appearance and biochemical test using standard procedure. Colonies that were Mannitol fermenter (golden yellow colonies) and β-hemolytic on SBA were subjected to Gram staining, catalase test and coagulase test. Colonies those are Gram positive cocci, catalase and coagulase positive were identified as *S. aurues* (7, 13, 23).

Figure.1 flow chart for identification of gram positive cocci



4.7.4Antibioticsusceptibility testing (AST)

Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disk diffusion test on Muller-Hinton agar. Three to five pure colonies of the test organism was taken using a sterile wire loop and emulsified in 2 ml of sterile physiological saline. The bacterial suspension turbidity was matched with 0.5 McFarland standard. Then a sterile cotton swab was dipped in to the suspension and squeezed free from excess fluid against the side of test tube and the test organism was uniformly seeded on the surface of Muller-Hinton agar. Using sterile forceps, the antibiotic discs were placed on agar plate by considering 24 mm distance between each disk and 15 mm from border according to clinical and laboratory standard institute (CLSI). The medium was incubated at 35 ^{o}C for 18-24 hours (5, 7).

In this study following antimicrobial discs with respective concentration were used: penicillin (10unit),Cefoxitin(30µg),Clindamycin(2µg),Azithromycin(15µg),Erythromycin(15µg),Trimethoprimsulfametoxazole(1.25/23.75µg),Tetracycline(30µg),Chloramphenicol(30µg)andGentamicin(10 µg).The antibiotic discs were selected based on recommendations from CLSI M100 S28(2018). .

MRSA is defined as any strain of *S. aureus* those had established resistance to beta-lactam antibiotics such as methicillin, oxacillin, cefoxitin and nafcillin (32). In this study screening of MRSA was done using cefoxitin disc (30µg), the strains those had zone of inhibition ≤ 22mm were considered as MRSA according to CLSI M100 S28(2018).

Interpretation of AST: Grades of susceptibility pattern will be interpreted by comparison of the zone of inhibition as indicated in the manufacturer's guide. The diameters of the zone of inhibition are measured using a metric scale reported as susceptible(S), intermediate (I), resistance(R)(5).

4.8. Data Quality Assurance

4.8.1. Pre-analytical

Socio-demographic characteristics and KAPs data were collected appropriately after getting consent and ascent and checked for completeness. This phase also includes selection of appropriate site and samples, collection, identification, and labeling (every sample container would have labeled with information's which includes: subject code, Date and time), handling,

and transportation of biological samples. All these steps were performed according to standard operating procedure (SOP) of the study site. Aseptic techniques were implemented in all the steps of specimen collection and inoculation on the culture media to minimize contamination.

4.8.2. Analytical

All materials, equipments and procedures was adequately controlled. All culture media was prepared according to the direction of the manufacturer. Culture media was tested for sterility and performance. Sterility of media was checked by incubating overnight at 37^{0} C. Performance of the media was tested by inoculation with bacterial species of known positive and negative reactions. ATCC (American type culture collection) 25923 *S. aurues* strain was used as control strain in antimicrobial susceptibility determination (5, 23, 24). Moreover; culture growth, biochemical test and antimicrobial susceptibility test results was confirmed by specially trained experienced microbiologists working in the microbiology unit of the study site.

4.8.3. Post-analytical

All of the extracted information (filled questionnaire, laboratory findings) was checked for legibility, completeness, consistency and recorded before entry to statistical tool. The data was also placed in secure location and stored in a CD as a backup. All laboratory isolates was stored as per the SOP of the study site.

4.9. Data analysis and interpretation

The finding was analyzed using descriptive statistics SPSS version 26, p value of ≤ 0.05 was considered as statistically significant. The variables from the demographic data and KAP data was performed and computed by using logistic regression analysis to determine the rate of *S.aureus* and MRSA colonization and other variables.

For each item of the scale, score of 1 was considered as a positive response and 0 represented as a negative response; then proportions of aggregate scores were calculated for items of knowledge. For attitude items; Likert scale of 1 to 5, with 1 being “strongly disagree” and 5 being “strongly agree” for positive questions; 5 being “strongly disagree” and 1 being “strongly agree” for negative questions was used. Regarding practice items scale of 1 to 3 with 1 stands for never, 2 stands for sometimes and 3 stands for always.

Good knowledge represents HCWs who score ≥ points on knowledge part of the questionnaires and poor knowledge for HCWs who score <5points on knowledge part of the questionnaires,

Positive attitude for HCWs who score ≥15 points on attitude part of the questionnaires and negative attitude for those HCWs who score <15 points on attitude part of the questionnaires and finally good practice stands for HCWs who score ≥11 points on practice part of the questionnaires and poor practice for HCWs who score <11 points on practice part of the questionnaires.(36,38)

4.10. Operational Definitions

Methicillin resistant *S.aurues* (MRSA): is defined as *S.aurues* strains those had zone of inhibition ≤ 22mm to cefoxitin disk.

Good knowledge: HCWs who score ≥ 5 points on knowledge part of the questionnaires (36,38)

Poor knowledge: HCWs who score < 5 points on knowledge part of the questionnaires (36, 38)

Positive attitude: HCWs who can score ≥ 15 points on attitude part of the questionnaires (36,38)

Negative attitude: HCWs who can score < 15 points on attitude part of the questionnaires (38)

Good practice: HCWs who can score ≥11 points on practice part of the questionnaires (36,38)

Poor practice: HCWs who can score < 11 points on practice part of the questionnaires (36,38)

4.11 Ethical considerations

Ethical clearance was obtained from Departmental Research and Ethics Review Committee (DRERC) of Medical Laboratory Sciences, from Addis Ababa Health Bureau Ethical Review Committee prior to data collection and permission letter was obtained from study hospital administration. The purpose and procedure of the study were explained for each study participant at study sites. Those who agreed to participate were asked to sign consent form. Written and informed consent was obtained. The confidentiality of the information collected was maintained by using code numbers for participants. Any participant who was not voluntary was not forced to be included as study subject.

4.12. Dissemination of the result

The findings of this study will be presented to department of Medical Laboratory Sciences for public defense. The result will be also communicated to Addis Ababa health Bureau and to the study site ZMH. Effort will be made to publish the findings in peer reviewed journals.

5. Results

5.1Sociodemographic characteristics

A total of 178 health professionals were included in the study. The age of study participants ranged from 24 - 58 years with mean age of 36.38 ± 8.179 years. Out of these, 103 (57.9%) were females and 75 (42.1%) were males. The mean number of their work experience was12.65 ±6.081 years.

5.2Prevalence of *Staphylococcus aureus and MRSA*

The prevalence of *S. aureus* and MRSA in this study was 12.9% (23/178) and 7.9% (14/178) respectively. Rate of MRSA among *S. aureus* was 60.9 % (14/23). The prevalence of MRSA among nurse, GP and midwife professionals were 10 (11.1%), 3(8.1%), and 1 (5.8%) respectively. The highest rate of MRSA was observed in surgical ward 2 (28.6%). The prevalence of *S.aureus* in females and males was 10.7% & 16.0%respectively, which shows that in this study; male participant were more colonized than females. Rate of colonization was slightly higher among aged group of the participants and in those who had more service years which is not statistically significant as shown in Table 2.

Table 2: Magnitude of *S. aureus* and *MRSA* in relation to socio-demographic characteristics of HCWs working at Zewditu Memorial Hospital Addis Ababa, Ethiopia, June 2021 (n= 178).

Variables	***R*esult**			**P Value**
	***S. aureus*(%)**	**MRSA (%)**	**Neg (%)**	
1.Gender				
Male	12(16.0%)	8(10.7%)	63(84.0%)	0.29
Female	11(10.7%)	6(5.8%)	92(89.3%)	
2.Age in years				
20-29	2(4.1%)	1(2.1%)	46(95.9%)	0.49
30-39	9(11.5%)	5(6.4%)	69(88.5%)	
40-49	7(18.4%)	5(13.2%)	31(81.6%)	
50-59	5(35.7%)	3(21.4%)	9(64.3%)	
3.Work experience				
≤5	1(4.5%)	1(4.5%)	21(95.5%)	0.57
6-10	4(6.1%)	3(4.5%)	62(93.9%)	

11-15	6(18.2%)	4(12.1%)	27(81.8%)	
16-20	7(17.5%)	4(10.0%)	33(82.5%)	
> 20	5(29.4%)	2(11.8%)	12(70.6%)	
4.Department				
Adult Emergency	0(0%)	0(0%)	13(100%)	0.94
OPDs	5(13.5%)	4(10.8%)	32(86.5%)	
Pediatrics ward	4(14.3%)	1(3.6%)	24(85.7%)	
Gyn OB	3(10.7%)	3(10.7%)	25(89.3%)	
Surgical ward	2(28.6%)	2(28.6%)	5(71.4%)	
Medical ward	1(11.1%)	0(0%)	8(88.9%)	
OR	3(16.7%)	3(6.7%)	15(83.3%)	
ICU	1(25.0%)	1(25%)	3(75.0%)	
Pharmacy	1(8.3%)	0(0%)	11(91.7%)	
Imaging	0(0%)	0(0%)	5(100%)	
Laboratory	3(17.6%)	0(0%)	14(82.4%)	
5.Profession				
GP	5(13.5%)	3(8.1%)	32(86.5%)	0.38
Nursing	12(13.3%)	10(11.2%)	78(86.7%)	
Midwife	2(11.8%)	1(5.8%)	15(88.2%)	
MLT	3(17.6%)	0(0%)	14(82.4%)	
Pharmacy technician	1(8.3%)	0(0%)	11(91.7%)	
Radiology technician	0(0%)	0(0%)	5(100%)	
6.Educational status				
Diploma	1(4.0%)	1(4.0%)	24(96.0%)	0.36
Bachelors	20(17.7%)	12(8.8%)	117(85.4%)	
Masters	0(0%)	0(0%)	12(100%)	
Others	2(50%)	1(25%)	2(50%)	

Key: *S.aureus* includes both *MSSA &MRSA*

Gyne OB, Obstetrics and Gynecology; OPDs, outpatients departments; OR, Operation room; ICU, Intensive care unit; GP, General practitioner; MLT, Medical laboratory technologist; Others, Specialists.

None of the socio-demographic characteristics mentioned in this study were found to be associated with *S.aureus* and MRSA colonization rate. Even though in binary logistic regression of age, service year and knowledge level of the participants were found to be significantly associated with *S .aureus and MRSA* colonization with OR value of 2.031 CI 95% (1.260-3.274) (p=0.004),1.671 CI 95% (1.181-2.364) (p=0.004) and 0.339 CI 95% (0.124-0.925) (0.035) respectively; but in multivariate analysis it was disproved that all of them are not a significantly associated with nasal colonization (P >0.050 as shown in Table below.

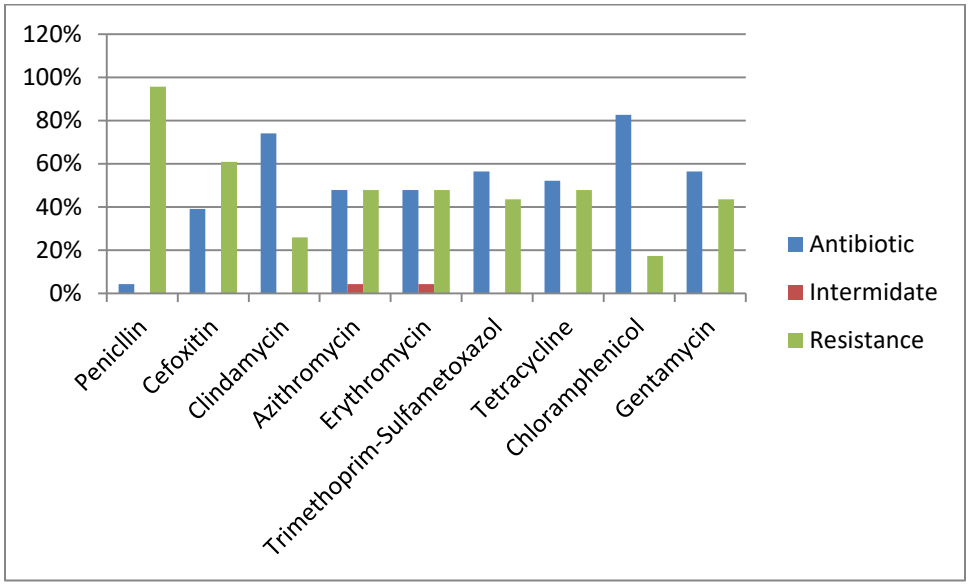
Table3; Bivariate and Multivariate analysis on association of *S.aureus* and KAP towards Socio demographic-characteristics of health care workers ZMH, 2021

Variables	S.E	P-value	Crude OR	Adjusted OR	95% CI for adjusted OR Lower	Upper
***S. aureus* colonization**						
Sex	0.448	.299	1.593	-	.662	3.836
Age	.574	.499	2.031	.679	.220	2.090
Service year	.418	.589	1.671	.798	.352	1.810
Department	.083	.941	.994	-	.845	1.169
Profession	.200	.367	.835	-	.564	1.236
Education	.410	.362	1.754	.688	.308	1.536
Knowledge	.562	.132	.339	3.632	.907	8.929
Attitude	.457	.755	1.154	-	.471	2.826
Practice	.525	.126	0.376	2.234	.798	6.251
Knowledge						
Sex	.425	.873	1.070	-	.465	2.461
Age	.235	.484	1.179	-	.743	1.870
Service year	.169	.703	1.066	-	.766	1.485
Department	.081	.343	1.080	-	.921	1.267
Profession	.227	.052	1.603	.643	.413	1.004
Education	.437	.021	2.880	.365	.155	.860
Attitude						
Sex	.308	.853	1.059	-	.579	1.935
Age	.167	.922	.984	-	.710	1.364
Service year	.122	.474	1.091	-	.860	1.385
Department	.184	0.564	1.367	.768	.651	3.906
Profession	.192	.322	1.700	.826	.567	1.205
Education	.332	0.081	1.768	.560	.292	1.074
Practice						
Sex	.305	.239	1.433	-	.788	2.607
Age	.371	.439	.800	1.333	.644	2.759
Service year	.013	.911	.854	.970	.575	1.638
Department	.081	.862	1.337	.747	.637	3.876
Profession	.176	.957	1.448	1.010	.715	1.426
Education	.277	.841	.946	-	.550	1.627

5.3 AST of the isolated *S. aureus*

AST pattern of the isolates was variable as twenty two (95.7%) of the isolates were resistant to penicillin, 14(60.9%) to cefoxitin, 11(47.8%) to Azithromycin, Erythromycin and Tetracycline, 10(43.5%) to Trimethoprim-sulfametoxazole and Gentamicin. Low resistance had showed to Clindamycin and chloramphenicol 6(26%) and 4(17.4%) as shown in the figure below.

Figure 2: Antimicrobial Susceptibility pattern of *S. aureus* by Disk diffusion



5.4. Assessment of knowledge, Attitude and practices towards MRSA

5.4.1Assessment of knowledge towards MRSA

HCWs Knowledge was assessed by focusing on ways of transmission and precautionary measures taken before being in contact with MRSA patients. Out of 178 participants, 150(84.3%) were within adequate knowledge range whereas 28 (15.7 %) showed poor knowledge about MRSA. When we see about effectiveness of hand hygiene; 155(87.1%) know that hand hygiene is effective in preventing MRSA. Concerning alcohol hand rub; 123(69.1%) know that alcohol hand rub is effective for MRSA. In terms of ways of MRSA transmission, the majority (149(83.7%)) indicated that these bacteria most often spread through HCWs hand. Only 105(59.0%) of the participant knows as asymptomatic carriers can spread MRSA. In terms of carriers treatment; 124(69.7%) knows as MRSA nasal carriers should be treated. On the other hand most of the participant 175(98.3%) had said glove should be worn in contact with MRSA patients.

Table4: Knowledge responses towards MRSA among HCWs at ZMH Addis Ababa, Ethiopia, June 2021(n=178).

Knowledge Items	Yes (%)	No (%)	Idon'tknow (%)
Have you trained infection prevention?	133(74.7)	45(25.3)	0(0)
Do you have any SOP for disinfection?	109(61.2)	69(38.8)	0(0)
Do you think hand hygiene is effective in preventing MRSA	155(87.1)	15(8.4)	8(4.5)
Do you think Alcohol hand gel is as effective as hand washing in reducing MRSA transmission?	123(69.1)	39(21.9)	16(9.0)
Do you think MRSA is transmitted via the hands of HCWs?	149(85.7)	18(10.1)	11(6.2)
Do you think Gloves should be worn when examining patients with MRSA?	175(98.3)	1(0.6)	2(1.1)
Do you think MRSA nasal carrier should be treated with nasal antibiotics?	124(69.7)	26(14.6)	28(15.7)
Do you think asymptomatic carriers can spread MRSA?	105(59.0)	46(25.8)	27(15.2)
Do you think your hospital has good infection control manag ement and practice?	63(35.4)	115(64.6)	0(0)

Adequate knowledge is probably an important factor in prevention of MRSA infection, the association between knowledge and MRSA status was shown in Table 5. Those with good knowledge were more likely less colonized with MRSA which is not statistically significant.

Table 5: Association of knowledge out come and *S. aureus* status among HCWs at ZMH Addis Ababa, Ethiopia, June 2021

Lab Result	**Knowledge outcome**		**P-value**
	Poor	Good	
S.A Positive	6 (21.4%)	17 (11.3%)	0.13
S.A Negative	22 (78.6%)	133 (88.7%)	
Total	28 (15.7%)	150 (84.3%)	

As indicated in table 6 below educated HCWs, gyne OB and medical ward staffs, GP and midwife professionals were more knowledgeable than other group of participants. However the difference did not reach at significance level except educational status of the participants which is statistically significant (p=0.02).

Table6: Association of knowledge out come and socio demographic characteristics among HCWs at ZMH Addis Ababa, Ethiopia, June 2021.

Gender	**Knowledge outcome**		**P-value**
	Poor N (%)	Good N (%)	
Male	12(16%)	63(84%)	0.873
Female	16(15.5%)	87(84.5%)	
Age group			0.484
20-29	11(22.9%)	37(77.1%)	
30-39	9(11.5%)	69(88.5%)	
40-49	6(15.8%)	32(84.2%)	
50-59	2(14.3%)	12(85.7%)	
Service year			0.703
<5	3(13.6%)	19(86.4%)	
6-10	12(18.2%)	54(81.8%)	
11-15	5(15.2%)	28(84.8%)	

16-20	6(15.0%)	34(85%)	
>20	2(11.8%)	15(88.2%)	
Department			0.343
Adult Emergency	3(23%)	10(77%)	
OPDs	9(24.3%)	28(75.7%)	
Pediatrics ward	6(21.4%)	22(78.6%)	
Gyne OB	1(3.6%)	27(96.4%)	
Surgical ward	1(16.7%)	6(83.3%)	
Medical ward	0(0%)	9(100%)	
OR	3(16.7%)	15(83.3%)	
ICU	1(25%)	3(75%)	
Pharmacy	1(8.3%)	11(91.7%)	
Imaging	1(20%)	4(80%)	
Laboratory	2(11.8%)	15(88.2%)	
Profession			0.052
MLT	2(11.8%)	15(88.2%)	
Nursing	23(25.6%)	67(74.4%)	
GP	0(0%)	37(100%)	
Midwife	1(5.9%)	16(94.1%)	
Radiology	1(20%)	4(80%)	
Pharmacy technician	1(8.3%)	11(91.7%)	
Educational status			0.021*
Diploma	9(36%)	16(64%)	
Bachelors	17(12.4%)	120(87.6%)	
Masters	2(16.7%)	10(83.3%)	
Others	0(0%)	4(100%)	

Key: Gyni OB, Obstetrics and Gynecology; OPDs, outpatients department; OR, Operation room; ICU, Intensive care unit; GP, General practitioner; MLT, Medical laboratory technologist; Others, Specialists ;∗statistically significant.

5.4.2. Assessment of Attitudes of HCWs towards MRSA

Attitude towards MRSA was evaluated using the Likert scale of 1 to 5, with 1 being "strongly disagree" and 5 being "strongly agree" for positive questions; whereas 5 being "strongly disagree" and 1 being "strongly agree" for negative questions . Since 6 questions were used, the summative value of this number of items ranged from 6 to 30. The value of perception ranged from 16 to 30 was labeled with positive attitude while the value from 6-15 was labeled as a negative attitude. More than 70% of participants agreed that MRSA was a national problem, but only 36% of participant agreed that people colonized with MRSA are danger to everyone they come into contact with. Similarly only 45.5% of participants were disagreed with the statement that says patient with an open wound infected with MRSA is a burden to hospital staff caring for them. On the other hand 28.7% of the participants believe that patients colonized with MRSA probably have poor personal hygiene, whereas only 38.2% of the participants were disagreed and 33.1% were neutral with this statement. Concerning contact with MRSA patient, more than 59% of participant disagreed with the statement" Hospital staff should spend as little time as possible with patients with MRSA infection. ".

Table 7: Attitude responses of HCWs towards MRSA at ZMH Addis Ababa, Ethiopia, June 2021 (n=178).

Attitude Item	Highly Disagree	Disagree	Neutral	Agree	Strongly Agree
MRSA is a worldwide problem.	2(1.1%)	19(10%)	31(17.4%)	74(41.6)	52(29.2%)
People colonized with MRSA are danger to everyone they come into contact with	14(7.9%)	64(36%)	36(20.2%)	53(29.8%)	11(6.2%)
A post-operative patient with an open wound infected with MRSA is a burden to hospital staff caring for them	10(5.6%)	71(39.9%)	40(22.5%)	41(23.0%)	16(9%)
Patients colonized with MRSA probably have poor personal hygiene	9(5.1%)	59(33.1%)	59(33.1%)	48(27%)	3(1.7%)
Hospital staff should spend as little time as possible with patients with MRSA infection	16(9.0%)	77(43.3%)	36(20.2%)	44(24.7%)	5(2.8%)
A patient with a MRSA infected wound does not make my job any more difficult	12(6.7%)	43(24.2%)	30(16.9%)	83(46.6%)	10(5.6%)

As indicated in table 8 below educated HCWs, staffs working in surgical ward, gyne OB, OR, Imaging and pharmacy department and midwife professionals had more positive attitude regarding *MRSA.*

Table 8: Association of Attitude out come and socio-demographic characteristics among HCWs at ZMH Addis Ababa, Ethiopia, June 2021

Gender	**Attitude out come**		**P-value**
	Positive N (%)	Negative N(%)	
Male	44(58.7%)	31(41.3%)	0.853
Female	59(57.3%)	44(42.7%)	
Age group			0.922
20-29	24(50%)	24(50%)	
30-39	50(64.1%)	28(35.9%)	
40-49	23(60.5%)	15(39.5%)	
50-59	6(42.9%)	8(58.1%)	
Service year			0.474
<5	10(45.5%)	12(54.5%)	
6-10	35(53.0)	31(47%)	
11-15	26(78.8%)	7(21.2%)	
16-20	22(55%)	18(45%)	
>20	10(58.8%)	7(41.2%)	
Department			0.564
Adult ER	3(23%)	10(77%)	
OPDs	19(51.4%)	18(48.6%)	
Pedi	8(28.6%)	20(71.4%)	
Gyne OB	24(85.7%)	4(14.3%)	
Surgical ward	7(100%)	0(0%)	
Medical ward	6(66.7%)	3(33.3%)	
OR	15(83.3%)	3(16.7%)	
ICU	2(50%)	2(50%)	

Pharmacy	10(83.3%)	2(16.7%)	
Imaging	4(80%)	1(20%)	
Laboratory	5(29.4%)	12(70.6%)	
Profession			0.322
MLT	5(29.4%)	12(70.6%)	
Nursing	47(52.2%)	43(47.8%)	
GP	23(62.2%)	14(37.8%)	
Midwife	14(82.3%)	3(17.7%)	
Radiology	4(80%)	1(20%)	
Pharmacy techn.	10(83.3%)	2(16.7%)	
Educational status			0.081
Deploma	12(48%)	13(52%)	
Bachelors	79(57.7%)	58(42.3%)	
Masters	8(66.7%)	4(33.3%)	
Others	4(100%)	0(0%)	

Key; Gyne OB, Obstetrics and Gynecology; OPDs, outpatients department; OR, Operation room; ICU, Intensive care unit; GP, General practitioner; MLT, Medical laboratory technologist; Others, Specialists .

5.4.3. Assessment of practices of HCWs towards MRSA

Practices of HCWs towards MRSA were assessed by asking 7 questions listed in table 9 below. Most of HCWs reported high rates of adherence to glove and gown during MRSA patient examination. Less than half of the participants follow SOP for disinfection and use proper disinfectant regularly. In terms of hand hygiene only 39.9% HCWs wash hands after each patient examination, 46.6% use soap and 38.8% use Alcohol hand rub as recommended.

Table 9: Self reported infection prevention Practice responses of HCWs towards MRSA at ZMH Addis Ababa, Ethiopia, June 2021 (n=178).

Practice Items	**Always**	**Sometimes**	**Never**
Do you wear gown during MRSA patient examination?	142(79.8%)	33(18.5%)	3(1.7%)
Do you wear glove during MRSA patient examination?	112(62.9%)	64(36.0%)	2(1.1%)
Do you follow SOP for disinfection?	85(47.8%)	88(49.4%)	5(2.8%)
Do you use proper disinfectant?	82(46.1%)	91(51.1%)	5(2.8%)
Do you wash your hands after each patient examination?	71(39.9%)	87(48.9%)	20(11.2%)
Do you use soap for hand washing?	83(46.6%)	90(50.6%)	5(2.8%)
Do you use Alcohol hand rub per patient examination?	69(38.8%)	88(49.4%)	21(11.8%)

The relation between practice outcome and laboratory findings are summarized in Table 10. As shown in the table, 16 (19.8%) of those participants with poor practice towards the bacteria were culture positive for *S. aureus*, which was relatively higher compared to those who had good practice. Those participants with good practice were relatively less colonized with *S. aureus*. The difference, however, did not reach statistically significant level except use of soap for hand washing which is statistically significant.

Table 10: Association of practice out come and *S. aureus* status among HCWs at ZMH Addis Ababa, Ethiopia, June 2021

Lab Result	**Practice outcome**		**P-value**
	Poor N (%)	Good N (%)	
S.aureus Pos	16 (19.8%)	7 (7.2%)	0.126
S.aureus Neg	65(80.2%)	90(92.8%)	
Total	81 (45.5%)	97 (54.5%)	

Association of practice out come and socio demographic factors were summarized in table below. Male participants, participants working in OR, ICU and pharmacy departments were more adhered to precautionary measures than others. On the other hand participants working in adult ER, OPDs, Pedi and surgical ward and nurses, midwives and lab professionals were less adhered to these measures.

Table11. Association of practice out come and socio demographic characteristics among HCWs at ZMH Addis Ababa, Ethiopia, June 2021

Gender	**Practice outcome**		**P-value**
	Good N (%)	Poor N (%)	
Male	38(50.7%)	37(49.3%)	0.239
Female	43(41.7%)	60(58.3%)	
Age group			0.439
20-29	22(45.8%)	26(54.2%)	
30-39	42(53.8%)	36(46.2%)	
40-49	14(36.8%)	24(63.2%)	
50-59	5(35.7%)	9(64.3%)	
Service year			0.911
<5	7(31.8%)	15(68.2%)	
6-10	38(57.6%)	28(42.4%)	
11-15	15(45.5%)	18(54.5%)	
16-20	15(37.5%)	25(62.5%)	
>20	6(35.3%)	11(64.7%)	
Department			0.862
Adult ER	3(23%)	10(77%)	
OPDs	11(29.7%)	26(70.3%)	
Pediatrics ward	7(25%)	21(75%)	
Gyne OB	17(60.7%)	11(39.3%)	
Surgical ward	3(42.9%)	4(57.1%)	
Medical ward	5(55.6%)	4(44.4%)	
OR	15(83.3%)	3(16.7%)	
ICU	3(75%)	1(25%)	
Pharmacy	9(75%)	1(25%)	
Imaging	3(60%)	2(40%)	
Laboratory	5(29.4%)	12(70.6%)	
Profession			0.957
MLT	5(29.4%)	12(70.6%)	

Nursing	34(37.8%)	56(62.2%)	
GP	22(59.5%)	15(40.5%)	
Midwife	8(47%)	9(53%)	
Radiology technician	3(60%)	2(40%)	
Pharmacy technician	9(75%)	3(25%)	
Educational status			0.841
Deploma	10(40%)	15(60%)	
Bachelors	66(48.2%)	71(51.8%)	
Masters	3(25%)	9(75%)	
Others	2(50%)	2(50%)	

Key; Adult ER, Adult accident and emergency; Gyne OB, Obstetrics and Gynecology; OPDs, outpatients department

6. Discussion

The overall nasal carriage of *S. aureus* in this study was 12.9% (23/178). This is supported by study done in Lahore (14.3%) [22], Nepal (15.7%)[23],Qazvin(16.1%)[26] ,Rwanda(17.1%)[42], Madagascar(11%)[28] , Adigrat and Wukro(12%)[13]. However, this report was lower than that of reported from Iran (34.2%)[5] , Gaza Strip (31.1%)[24] Pakistan(24%)[25], India(36.7%)[3], Uganda(28.8%)[30], Kenya(34.4%)[31], Dessie(28.8%)[34], Mekelle (20.3%)[19], Gondar (34.4%)[7] and Jimma (22.1%)(33).The total prevalence of MRSA in this study was 7.9%(14/178). This was similar with reports from Lahore(5.2%)[22], Iran(7.8%)[5], Pakistan(9.3%)[25] ,Rwanda(5.7%)[42] ,Jimma(8.4%)[33] and Adigrat and Wukro (5.8%) [13] However, it was lower compared to the study revealed in Ethiopia, Mekelle (14.1%) [19], Dessie (12.7%) [34] and Gondar(13.5%)[7], Kenya (18.9%)[31], Uganda(13.4%)[30], Gaza Strip (25.5%) [24] and Pakistan (22%) [2]. On the other hand, our finding was higher than reports from Australian (3.4%)[27], Europe & America (1.1%)[21], Nepal (3.4%)[23], India(2.93%)[3] and Madagascar(1.3%)[28]. This variations in colonization rate among different study areas might be due to difference in rate of patient load, study period, microbiological methods (sample size, culture media used, MRSA identification method), antimicrobial policy, sanitation status of the health institutions (availability of sanitary supplies including sink, water, soap, bleach etc.), commitment level of infection prevention control among hospitals, and awareness of the HCWs about MRSA may contribute to the difference.

In this study, MRSA carriage was relatively higher among nurse professionals 11.2%(10/89) followed by doctors 8.1%(3/37) and midwives 5.5%(1/18).This is consistent with study conducted in Ethiopia, Dessie [34], Mekelle[19],Adigrat and Wukro [13],Gaza Strip [24] ,Pakistan[2],and Australia [27]. But our report is not similar to study conducted at South Punjab Pakistan which had reported higher carriage among laboratory technicians followed by nurse professionals, medical doctors and pharmacy professionals [25]. In addition this report is also inconsistent with report from Kenya in which colonization rate was higher among doctors (27.9%) than nurses (16.3%) [31].

Rate of MRSA carriage was particularly high among surgical ward 28.6%(2/7) followed by Intensive Care Unit 25%(1/4), Operation room16.7%(3/18),OPDs 10.8%(4/37), gyneobs 10.7%(3/28) and pediatrics department 3.6%(1/28). This result is comparable with corresponding study conducted at Gaza Strip [24], Nepal [23], Qazvin hospitals, Iran[26] and Adigrat [13].

However it is inconsistent with study conducted in Uganda which had reported 50% colonization rate among laboratory department [30].This variation in colonization rate among different department and profession might be because of the frequent direct physical contact of doctors and nurses with patients and increased workload in these wards. In addition, carriage rates might be influenced by poor adherence of study participants to standard precautionary measures and variation of the hospitals set up may results in this difference.

Antimicrobial resistance is an important problem in the management of patients with infectious diseases. Interestingly, Clindamycin and chloramphenicol were effective against MRSA isolates in many countries of the world. In this study, *S.aureus* isolates were resistant to pencillin(95.7%),cefoxitin(60.9%),Erythromycin(47.8%),Tetracycline(47.8%),Azithromycin(47.8%),Cotrimoxazole(41.7%),Gentamicin(41.7%),Clindamycin(26%)andChloramphenicol(17.4%) which agrees with the previous studies conducted in Ethiopia and also concordant with many studies conducted all over the world (5,7,13,19,22,23)**.**

In this study over all, HCWs had average knowledge towards MRSA. Out of the 178 respondents, 150(84.3%) were within good knowledge range whereas 28 (15.7 %) had showed poor knowledge regarding MRSA which is consistent with study conducted in Egypt (78.6%)[43], Gondar(90%)[45] and Dessie (86.4%)[46]. However this report was higher than report from Tobago (20.3%) [37] and Rwanda (51.2%)[42]. Of the participants 87.1% knew that hand hygiene is effective in preventing MRSA which agrees with study conducted in other parts of the world (35, 36, 38, 42). When we come to alcohol hand rub effectiveness for MRSA reduction; 69% of the respondents knew that alcohol hand rub is effective for MRSA prevention which is higher than report from United State (34.6%), Mississippi (48.8%) and Palestine (5.7%) [35,36,40].Concerning mechanism of MRSA transmission, majority (83.7%) of the participants were able to identify that they are often the ones who spread MRSA through their hands and 98.3% of them had said wearing of gloves when examining patients with MRSA should be mandatory which is concordant with those result summarized in literature review[35, 36,38,39].When we see about MRSA transmission by asymptomatic carriers , only 59.0% of the participants knows as asymptomatic MRSA carriers can spread MRSA which is similar with the report of study conducted in Palestine[40]. However this result is lower than reports from United States, Korea, Mississippi and Malaysia [35, 36, 38, 39]. In terms of carriers treatment, only

69.7% of the respondents knows as MRSA nasal carriers should be treated which is lower than reports from Nigeria but higher than reports from other countries [36, 41].

In our study knowledge was relatively higher among educated participants which is in line with study conducted in Gondar and Debre Markos[45,47]. In addition GP and midwife professionals were more knowledgeable than others which is inconsistent with reports from other countries [36,39]. On the other hand; in our study there is no much difference between other socio demographic factors which is inconsistent with report of other studies [45,47]. This might be so because healthcare workers with higher educational level might have acquired essential information, hence they might acquire infection prevention course

According to this study, overall Participants attitudes towards MRSA was not enough which is almost half of the respondents (42.1%) had negative attitude towards MRSA, whereas only 57.9% of the participants had showed positive attitude regarding MRSA. This result is supported by the report of study conducted in Gondar (57.2%) [45]. However, this result was higher than reports from Trinidad & Tobago [37]. On the other hand this report was lower than reports from India, Egypt, Gondar and Dessie [3, 4, 46, 51]. Health care workers in this study were more likely to feel that patients with MRSA were a burden to them; probably they had poor personal hygiene which is discordant with study conducted in Mississippi [36].On the other hand more than half of the participant took up more of their time with MRSA patients which is similar to reports in the literature review [38]. More than 70% of the participants agreed that MRSA was a national problem which is supported by other studies summarized in literature review [36,40].

In our study educated participants and staffs working in surgical ward had more positive attitude which is in line with report of other countries [45, 47].

Generally, almost half of HCWs (54.5%) were not compliant to MRSA protocols in this study which is supported by reports from Trinidad & Tobago (44%) [37]. However this is much more less than study conducted in Malaysia (94.5%)[38] and Egypt (80.8%)[51] while it was higher than reports from Gondar(36%)and Dessie(23%) [45,46]. Only 62% of the participants stated that they always wear gloves during examination of MRSA patients, which is similar with reports from Palestine[40]. However; this report is lower than report from Korea and Malaysia [36,38,39].On the other hand most of HCWs (79.8%) reported high rates of adherence to gown in contact with MRSA patients which is concordant with reports from Malaysia but higher than reports from Palestine and Mississippi [36,40]. In terms of hand hygiene only 39.9% of HCWs

had said they wash their hands after each patient examination, 46.6% use soap and 38.8% use alcohol hand rub as recommended which is much lower than report of other studies [36, 38, 39, 40].

7. Limitation

- PCR techniques could not be used for genotypic identification of *S. aureus* strain.
- Low sample size
- Only disc diffusion method was used for susceptibility testing.

8. Conclusion

The overall prevalence of MRSA and S.aureus in the study area was found to be7.9% & 12.9%. The carriage rate of MRSA was higher among nurses and in surgical wards. Rate of colonization was slightly increased with the age and service year of the participant. Clindamycin and chloramphenicol had higher sensitivity rate for MRSA and S. aureus, so it could be used as a treatment of choice. Healthcare workers in this study had sufficient knowledge towards infection prevention, but their attitude and implementation of safe practices were limited. Educational level of HCWs was significantly associated with knowledge of the participant. Therefore, provision of continual trainings are recommended. Additionally, hospitals should provide infection prevention facilities and supplies, continuous water supply, hand washing sinks and all necessary PPE to improve infection prevention practices of healthcare workers.

9. Recommendation

Based on the finding of these study the following are recommended

- HCWs should be regularly screened in order to have an early warning information about antimicrobial resistant pathogens in the hospital.
- Colonized HCWs should be treated to reduce risk of transmission
- MRSA patients should be isolated as it has critical role in prevention and control this infection.
- HCWs should be periodically educated and trained about MRSA infection prevention and control

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Annex

Annex I. General information for the study participants (English version)

Introduction

My name is Lensa Makulo and I am MSc student of Addis Ababa University, School of Medical Laboratory Sciences. I am doing research entitled nasal carriage rate of *S.aurues* and its antimicrobial susceptibility pattern among health care workers at Zewditu memorial hospital Addis Ababa, Ethiopia. With advances in health care system, threat to Hospital Acquired Infections (HAIs) still remains high/low. Currently hospital acquired MRSA infection continue to affect hospitalized patients and results in morbidity, mortality and additional costs as several study indicates. So this study will indicate the nasal carriage rate and drug resistance patterns of *S.aurues* among HCWs in Zewditu memorial hospital. In addition this study may also used to determine associated risk factors and knowledge and attitude of staff on transmission of infection caused by this organism.

What is the reason of this study? The objective of this research is to determine the prevalence of nasal *S.aurues* and antimicrobial resistance patterns among HCWs in Zewditu memorial hospital Addis Ababa, Ethiopia. All consented HCWs who agree to participate in the study will give a nasal swab for culture as per the standard and they also interviewed about some clinical information.

Will the information be confidential? All the data obtained will be kept confidentially; only the study personnel will have accesses to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

What are the costs? All the investigations performed for the participants of this study will be free of charge.

What about compensation? You will not be compensated for your participation in this study but you and your patient may benefit from the study because more intensive investigations will be done which may allow early detection of the warning information regarding the spread infection.

What about my rights to decline participation or withdraw from the study?

Your participation in this study is purely voluntary, and you may stop the participation or you may refuse to answer some of the questions if you feel uncomfortable. You are free to participate in this study or you can withdraw your consent anytime, which will not involve any penalty or loss of benefits to which you are entitled.

What about the harm which may happen in the study?

This research involves taking of nasal swab from HCWs selected in the study. There are no major risks to HCWs and will not cause any harm to the hospital.

Assurance of the principal investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of reports for all stakeholders of the research project.

Lensa Makulo (PI): signature _____Date_____

Note: if you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: Lensa Makulo: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

E-mail: lensmak82@gmail.com: Tele: +251913941382

Annex II. Information sheet (Amharic version)

መግቢያ

ስሜሌንሳማኩሎበአዲስአበባዩኒቨርስቲበጤናሳይንስኮሌጅበሜዲካልላቦራቶሪትምህርትክፍልማስ ትሬትዲግሪተማሪስሆንበዘውዲቱሆስፒታልየሚሰሩጤናባለሙያዎችከአፊንጫቸዉፈሳሽበመውሰ ድበብዛትጤናባለሙያዎችላይየሚገኝናበሆስፒታልየሚታከሙህሙማንንየሚያጠቃውንባክቴሪያ ላይምርምርእሰራለሁ፡፡ስለዚህይህ ጥናትበዘውዲቱሆስፒታል ጤናባለሙያዎችላይያለውን የባክቴሪያ መጠን ለማየት ይረዳናል፡፡

የጥናቱምክንያት

ይህበሳይንቲፊክመጠሪያውS.auruesየሚባልስሆንብዙጊዜበሆስፒታልየሚታከሙህሙማንንለሌ ላበሽታናሞትከመዳረጉምበላይለብዙወጭህሙማንንእንደሚዳርግበብዙጥናቶችተረጋግጧል፡፡ስለ ዚህይህጥናትበዘውዲቱሆስፒታልጤናባለሙያዎችላይያለውንየባክቴሪያመጠንለማየትይረዳናል::ከ ተስማሙበዚህጥናትላይለመሳተፍፈቃደኛከሆኑስለሁኔታውበደንብእንዲያውቁይደረጋል፡፡

የጥናቱሚስጥራዊነት

የተገኘዉመረጃሁሉበሚስጥራዊነትበተቆለፈክፍልወይምሳጥንዉስጥበማስቀመጥየጥናቱባለበት ብቻእንዲገኘዉይደረጋል፡፡በምርመራወቅትየተለየኮድበመጠቀምየተመርማሪዉውጤትእንዳይታ ወቅእንከላከላለን፡፡

የክፍያጉዳይ

በጥናቱላይበሚደረጉሁሉምአይነትየላቦራቶሪምርመራዎችወጭከክፍያነፃሆኖበተመራማሪዉይሸ ፈናል፡፡

የማካካሻጉዳይ

በጥናቱላይስለተሳተፉምንምዓይነትማካካሻአያገኙምነገርግንህሙማኑከሚደረገዉጥልቅምርመራ በሽታዉበጊዜተገኝቶለትበፍጥነትይታከማል፡፡

ከጥናቱላይያለመሳተፍናየመገለልመብትጉዳይ

እርሶበጥናቱላይየተሳተፉትሙሉበሙሉበፈቃደኝነትላይየተመሰረተነዉ፡፡ስለዚህእርሶበማንኛዉም ሰዓትበጥናቱላይመሳተፍማቆምይችላሉ፡፡ያለመሳተፍምመብትዎነዉ፤አንዳንድጥያቀዎችካልተመ ቾትያለመመለስምመብትአለዎት፡፡ይህንበማድረግዎምንምአይነትቅጣትአያስከትልቦዎትም፤ማግ ኘትየሚገባዎትንሁሉንምነገርአያሳጣም፡፡

ከጥናቱ ጋር የተያያዙ ተጓዳኝ አደጋ ጉዳይ

ናሙናበሚወሰድበትጊዜከሚሰማዉያለመመቾትበስተቀርምንምአይነትየከፋጉዳትበጥናቱምክንያ ትአይከሰትም፡፡

የተመራማሪዉማረጋገጫ

እኔከስርፊርማየንያስቀመጥኩትሳይንሳዊጥናቱንበግብረገብበተሟላመልኩእንደማካሀድናየጥናቱ ንርፖርትለሚመለከታቸዉባለድርሻአካላትእንደማቀርብበፊርማየአረጋግጣለሁ፡፡

ሌንሳማኩሎ (ዋናተመራማሪ)፡ፊርማ---------- ቀን

ማስታወሻ፡

ስለጥናቱማንኛዉምጥያቄካለዎትበሚቀጥለዉአድራሻነፃሆነዉይጠይቁን፡

የዋናተመራማሪአድራሻ፡ ሌንሳማኩሎ

በአዲስአበባዩኒቨርሲቲ፣የጤናሳይንስኮሌጅ፣የህክምናላቦራቶሪትምህርትክፍል፤አዲስአበባኢትዮጲ ያ፡፡

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Annex III. Consent form for Health Care Workers (English version)

I have read the information about the study which plans to determine HCWs nasal carriage rate of *S.aurues* and antimicrobial drug resistance pattern in Zewditu memorial hospital, Addis Ababa Ethiopia. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. The objective and the application of the study were briefly explained to me. I voluntarily consent that I would participate in this study.

I have been well informed of my right to refuse information, decline to cooperate and dropout of the study if I want.

Print name of participant, date and signature of participant

_____ ____ /____ /____ (dd/mm/yy) _____

Phone number

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

Annex IV. Consent form for health care workers (Amharic version)

በዘውዲቱሆስፒታልየሚሰሩጤናባለሙያዎችከአፊንጫቸዉፈሳሽበመውሰድበብዛትጤናባለሙያዎችላይየ
ሚገኝናበሆስፒታልየሚታከሙህሙማንንየሚያጠቃውንባክቴሪያላይምርምርእሰራለሁስለሚለዉምርምር
በቂመረጃአግንቻለሁ።የጥናቱአላማናአተገባበርበመጠኑተገልፆልኛል።በጥናቱላይያለመተባበርያለመሳተፍና
ከተሳተፍኩምበኋላየማቋረጥመብትእንዳለኝተረድቻለሁ።እኔባለመተባበርምክንያትምንምአይነትበደልበ
ጤናተቋሙእንደማደርስብኝተገልፆልኛል።ስለዚህያለዉንሁኔታሙሉበሙሉተረድቼበሙሉፈቃደኝነትበጥና
ቱላይለመሳተፍናሙናእንዲወሰድምረምራእንዲከናወንስልፈቃደኛመሆነንአረጋግጣለሁ።የተወሰደውናሙና
በሽታአምጭተዋሲያንንለመመርመርናተዋሲያኑለመድሃንትያላቸዉንምላሽለማወቅእንደሆነአዉቄተስማ
ምቻለሁ።ጥያቄዎችንየማቅረብእዲልአግኝቻለሁጥያቄየምእኔበሚረዳዉቋንቋባግባቡተመልሶልኛል።ከዚህ
ምበተጨማሪየናሙናዎቹዉጤትባስፈለገኝጊዜማግኘትእንደሚችልአዉቀያለሁ።

ስምና ቀን ፅፈዉ ይፈርሙ

ስም----------ቀን -------/-----/----------ፊረማ ----------

የተመራማሪዉስምናፊርማ

ስም _____ ቀን_____ፊረማ_____

የተሳታፊዉ ኮድ፡_____

Annex V. Questionnaire

I. **Socio-demographic characteristics HCWs**

This information is very valuable to us. The information you provide is completely voluntary and anonymous. We value any information you will be able to provide for us.

1. Participant code: _ _ _ _ _
2. Date of interview (dd/mm/yyyy) /_ __ /_ __/
3. Department _____
4. Profession_____
5. Participant address(phone no)_____signature_____
6. Data collector name _____signature_____
7. Age
8. sex male □ female □
9. year of service 1-5 years □ 6-10 years □ 11-15 years □ 16-20 years □ >20yrs
10. Educational Status:

diploma □ bachelors□ masters □ other specify_____

II. Other clinical characteristics

11. History of recent antibiotic use Yes □ No □

If yes mention antibiotic used and time taken_____

12. History of recent nasal surgery or bleeding Yes □ No □

III. Knowledge characteristics

1. Have you trained infection prevention? Yes □ No □ I doṅt know □

2. Do you have any standard operating procedure (SOP) for disinfection? Yes □ No □I doṅt know □

3. If question no 2 is yes; look for standard criteria full filled? Yes □ No□

4. Do you think hand hygiene is effective in preventing MRSA spread? Yes □ No Idoṅt know □

5. Do you think Alcohol hand gel is as effective as hand washing in reducing MRSA transmission? Yes □ No□ I doṅt know □

6. Do you think MRSA is transmitted via the hands of healthcare workers? Yes □ No Idoṅt know □

7. Do you think Gloves should be worn when examining patients with MRSA? Yes □ No□ Idoṅt know □

8. Do you think MRSA nasal carrier should be treated with nasal antibiotics? Yes □ No□ Idoṅt know

9. Do you think people who carry MRSA but do not have symptoms can spread MRSA? Yes □ No □ I doṅt know

10.Do you think your hospital has good infection control management and practice? Yes □ No □ I doṅt know

IV. Attitude characteristics

Note: 1-HighlyDisagree 2-Disagree 3-Neutral 4-Agree 5-HighlyAgree

1. MRSA is a worldwide problem.	
2. People colonized with MRSA are a danger to everyone they come into contact with	1 2 3 4 5
3. A post-operative patient with an open wound infected with MRSA is a burden to hospital staff caring for them	1 2 3 4 5
4. Patients colonized with MRSA probably have poor personal hygiene	1 2 3 4 5
5. Hospital staff should spend as little time as possible with patients with MRSA infection	1 2 3 4 5
6. A patient with a MRSA infected wound does not make my job any more difficult	

V. Practice characteristics

1. Do you wear gown during MRSA patient examination? Always □sometimes□ never□
2. Do you wear glove during MRSA patient examination? Always□ sometimes□ never□
3. Do you follow SOP for disinfection? Always□ sometimes□ never□
4. Do you use proper disinfectant? Always□ sometimes□ never□
5. Do you wash your hands after each patient examination? Always □sometimes □never□
6. Do you use soap for hand washing? Always□ sometimes□ never□
7. Do you use Alcohol hand gel per patient examination? Always□ sometimes□ never□

Annex VI: Standard operating procedures (SOPs)

SOPs that are required for media preparation, reagent preparation, specimen collection and laboratory investigation are described below. In general manufacturers' instructions should be followed to prepare, store and perform quality control for each regents and medium used in this study.

SOP for Blood agar media preparation

Purpose: This procedure provides instructions how to prepare Blood agar media.

Principle: Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Blood Agar Base. The medium contains sodium chloride for the osmotic balance. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic microorganisms. Sheep blood gives best results for *S.aurues*. Hemolytic patterns may vary with the source of animal blood or type of base medium used. The low pH helps in stabilization of red blood corpuscles and favors the formation of clear hemolysis zone.

Abbreviation:

BA= blood agar

Materials, Supplies and equipment

• Blood agar base powder

• Weighing paper

• Distilled water

• Spatula

• Sterile sheep blood

• Refrigerator

• Balance

• Autoclave

• Hot plate

• Bunsen burner

• Distiller

• Dispenser

• Graduated cylinder

• Flask

• Test tube

• PH meter

Procedure:

1. Weigh and Suspend 40grams of powder in1littr distilled water

2. Mix thoroughly and heat to boiling to dissolve the medium completely with frequent agitation

3. When cool adjust the ph to 7.3

4. Autoclave at 15 lbs pressure at (121oc) for 15 minute

5. Cool the medium at 50 ^{0}C

6. Add 50ml of sheep blood and mix with gentle rotation.

7. Dispense 20ml of the solution in to sterile Petri dish

8. Allow the medium to solidify label with date and store at 4 ^{0}C

Limitations: Blood agar is not a selective media so we couldn't differentiate microorganisms from the agar.

Clinical utility: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to observe the forms of hemolysis from pathogenic microorganisms.

SOP mannitol salt agar preparation.

PURPOSE:This procedure provides instructions how to prepare mannitol salt agar.

PRINCIPLE: Mannitol Salt Agar is a nutritive medium due to its content of peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species.

Abbreviation:

MSA: Mannitol Salt Agar

Materials, Reagents, supplies and equipment

- MSA powder
- Balance
- Spatula
- Weighing paper
- Distilled water
- Autoclave
- Hot plate
- Bunsen burner
- Distiller

- Water bath
- Graduated cylinder
- Sterile Petri dish

PROCEDURE

1. Suspend 111grams of MSA powder in1liter distilled water.

2. Heat to dissolve the medium completely.

3. Autoclave at 15 lbs pressure at (121^{o}c)

4. Cool and pour 20ml in to sterile Petri dish

5. Store at 2-8^{o}cup to usage

Limitation: Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Clinical Utility: Mannitol Salt Agar is used for the selective isolation and enumeration

of staphylococci from clinical and non clinical materials

SOP for gram staining

PURPOSE: The gram staining reaction is used to help identify pathogens in specimens and cultures by their gram reaction and morphology.

PRINCIPLE: Difference in gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of gram positive and gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violate and treatment with iodine, the dye-iodine complex is easily removed from the more permeable cell wall of gram negative bacteria but not from the less permeable cell wall of gram positive bacteria. Retention of crystal violate by gram positive organisms may also be due in part to the more acidic protoplasm of these organism binding to the basic dye(helped by the iodine).

Reagents

1. Crystal violate (or gentian violate)

2. Lugol's iodine

3. Aceton-alcohol decolorizer (or ethanol-iodine)

4. Safranin(counter stain)

Reagent Stability and storage: Store at room temperature in a foil-covered bottle or brown bottle to protect solution from light.

Materials, supplies and equipment

• Slides.

• Wooden applicator sticks

• Immersion oil

• Disposable glove

• Light Microscope

• Staining rack/jar

• Timer

• Staining jars

• Racks for drying slides

Storage: Fresh colonies at room temperature

Sample retention: Samples are discarded after the test has been done.

QUALITY CONTROL:

Before doing gram staining test check new batches of stain and reagents for correct staining reactions using a smear containing known gram positive and gram negative organisms.

PROCEDURE

1. Fix the dry smear

2. Cover the fixed smear with crystal violates stain for 30 -60 seconds.

3. Rapidly wash off the stain with clean water

4. Tip off all the water, and cover the smear with lugol's iodine for 30 -60 seconds.

5. Wash off the iodine with clean water.

6. Decolorize rapidly (few seconds) with acetone-alcohol. Wash immediately with clean water

7. Cover the smear with neutral red stain/safranin for 2 minutes.

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean; place it in a draining rack for the smear to air-dry.

10. Examine the smear microscopically, first with 10X objective to check the staining and to see the distribution of material, and then with oil immersion objective to report the bacteria and cells.

Result interpretation:

Gram positive bacteria ……. Dark purple

Morphology of the bacteria ……. …...Cocci,

Limitation:

1. Gram positive organisms may lose their ability to retain crystal violate and stain gram negatively because of:

1.1 Cell wall damage due to antibiotic therapy or excessive heat-fixation of the smear.

1.2 Over-decolonization of the smear.

1.3 Use of an iodine solution which is too old, i.e. yellow instead of brown in color (always store in a brown bottle or other light opaque container)

1.4. Smear has prepared from an old culture.

2. Gram negative organisms may not be fully decolorized and appear as gram positive when a smear is too thick.

Clinical utility: The Gram's stain is used to classify bacteria on the basis of their forms, sizes, cellular-morphologies, and gram reaction.

SOP for Catalase

Purpose: This procedure provides instructions to detect the production of catalase by bacteria.

Principle: Catalase is an enzyme that breaks hydrogen peroxide (H2o2), a toxic metabolic by products of aerobic and facultative anaerobic bacteria into nontoxic products water (H2O) and oxygen (O2). H2O2 is toxic to cells. It is highly reactive molecule that damage cell components. So the bacteria living in presence of oxygen produces enzyme catalase that breakdown H2O2 into H2O and O2.

Requirements:

Pure culture of staphylococcus

3% Hydrogen peroxide

A glass slide/test tube

Procedure

Slide test:

1. Prepare a fresh hydrogen peroxide 3% solution

2. Place a drop of 3% H2O2 on opposite ends of a clean grease free glass slide with the help of dropper

3. Transfer as small portion of both cultures onto drop of 3% H2O2 with the help of a sterile glass rod

4. Examine for immediate bubbling of O2

Note: slide test is not recommended because of risk of contamination from active bubbling

Tube test:

1. Pour 2-3ml of 3% hydrogen peroxide into a test tube

2. Transfer the culture with the help of glass rod and immense into H2O2 solution

3. Observe for immediate bubbling

Result interpretation:

Staphylococcus spp: give bubbling of gas, catalase positive

Limitation: Do not use wire loop to transfer the culture as H2O2 may reacts with iron to give false positive; H2O2 is toxic to human so handle carefully; observe the result within 30 seconds; culture should not be more than 24 hours old

SOP for Coagulase

Purpose

This procedure provides instructions for Coagulase test

Principle

Coagulase test is used to differentiate *Staphylococcus aureus* from other commonly isolated staphylococci. Coagulase is a thermostable thrombin-like substance that activates fibrinogen to form fibrin, resulting in a fibrin clot. This is demonstrated in the test tube by the formation of a clot when plasma is inoculated with the staphylococcus. The substance is known as free coagulase, since it is liberated by the cell. In most, but not all, *S. aureus* organisms, fibrinogen binding cell surface receptor is also present in the cell wall, called “bound coagulase” or

"clumping factor." Clumping factor is demonstrated by the ability of the organism to act directly on the fibrinogen on the plasma to clump it in a slide method.

Requirements

Rabbit plasma in EDTA

Sterile inoculating loop or applicator sticks

Glass or plastic tubes (12 x 75mm), and

Sterile deionized water

Procedure

<u>Tube method</u>:

1. Add 0.5ml of Rabbit's plasma into a test 12 x 75mm tube.

2. Inoculate the tube with several colonies of the test organism.

3. Incubate at 35˚C for up to 4 hours and observe hourly for clot formation. Do not agitate the tube; rather gently tip to observe the clot. If test is negative after 4 hours continue to incubate the test for 24 hours.

4. After 24 hour incubation, observe for clot formation.

5. Record results

Notes:

To avoid misidentifications, only perform this test on classic-looking, white to yellow, creamy, opaque, hemolytic colonies of gram-positive cocci in clusters that are catalase positive.

MRSA can be deficient in bound coagulase, which results in negative slide test.

Coagulase testing cannot be performed from growth on mannitol salt agar.

Interpretation

Positive test - complete clot formation or any degree of clot formation

Negative test - lack of clot formation

Reporting of results

Report as *Staphylococcus aureus* if the tube test is positive, catalase positive and a gram-positive coccus in clusters.

Limitation: Rabbit plasma is preferable than human plasma; as it gives better clotting, free from inhibitors and safe. Human plasma contains sodium citrate as anticoagulant, and some citrate utilizing bacteria such as Enterococcus faecalis which can cause clotting. False positive or false negative can occur if the plasma is not sterile.

The test for clumping factor is rapid but requires several colonies and the factor is not present in all *S. aureus* strains. In addition, clumping factor can be present in other species, namely, *S. lugdunensis* and *S. schleiferi.*

Sop for Antibiotic sensitivity testing

Purpose: This procedure provides instructions to determine the drug sensitivity pattern of bacteria using Kirby Bauer disk diffusion method.

Principle: The antibiotic will diffuse in a radial manner from the disc and will inhibit bacterial growth around it

Abbreviation:

ATCC= American Type Culture Collection

CLSI= Clinical and laboratory standards institute

Material, Supplies, reagent and equipment

• Muller hinton agar

• Normal saline

• Test tube

• Wooden applicator sticks with cotton

• Antimicrobial disks

• Safety cabinet

• Bunsen burner

• Incubator

• Measuring caliper

• Vortex

• Candle jar

Limitations: Comparing the inoculums turbidity with the standard McFarland is subjective.

Sample retention: Samples are discarded after the test has been done.

Quality Control

Control	Stability	Frequency	Preparation Y/N
ATCC	6 weeks at room temperature	Weekly sub-cultured	Y

Control Preparation

1. Reconstitute the lyophilized standard strain in to 1ml TSY broth or Normal Saline.

2. Inoculate in to BAP & MSA

3. Incubate for 16 – 24 hrs at 35 – 37 ^{0}C

4. Perform sensitivity test

5. Compare the sensitivity result with CLSI guideline.

Note: If the results are out of expected value, repeat the test and take corrective action.

Procedure:

1. Prepare pure colony suspension in to normal saline equivalent to 0.5 Mcfarland standards.

2. Streak on appropriate media the entire surface.

3. Select antimicrobial agents according to the CLSI guideline & Put the disc on the plate aseptically

4. Incubate for 16 – 24 hrs at 35 +/- 2oC

5. Measure zone of inhibition and interpret the result based on CLSI break point.

Result interpretation:

Susceptible (S): The 'susceptible' category implies that isolates are inhibited by the usual achievable concentration of antimicrobial agent when the recommended dosage is used for the site of infection.

Intermediate (I): The 'intermediate' category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated or when a higher than normal dosage of a drug can be used. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margin.

Resistant (R): The 'resistant' category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistant mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

Limitation: The response to antimicrobial therapy in vivo may not always reflect results in vitro.

Clinical utility: To detect in vitro the relationship between an organism and an antibiotic to predict the failure or success of therapy in vivo (in patient).

SOP for Muller Hinton media preparation

Purpose: This procedure provides instructions to prepare Mueller Hinton agar.

Principle: Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Abbreviation:

MHA= Mueller Hinton Agar

RT= Room Temperature

PABA =Para aminobenzoic acid

Materials, Supplies and equipment

• MHA powder

• Distilled water

• Flask

• Petri dish

• Graduated cylinder

• Balance

• Distiller

• Bunsen burner

• Autoclave

• Hot plate

• PH meter

Procedure:

1. Suspend 38 gm of MHA powder & transfer in to a flask containing 1000 ml of distilled water.

2. Boil until the powder completely dissolved

3. Autoclave at 1210c for 15 minute.

4. Adjust PH at 25oc is 7.3 +/- 0.2.

5. Mix well and dispense aseptically in to sterile Petri dish.

Clinical Utility: MHA is used in antimicrobial susceptibility testing by the disk diffusion method.

Declaration

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

M.Sc. candidate: Lensa Makulo (BSc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors: Kassu Desta (MSc, PhD candidate)

Regasa Diriba (MSc.)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.