ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

NASAL COLONIZATION OF METACILLIN-RESISTANT *STAPHYLOCOCUS AURUS* AMONG SURGICAL WARD ADMITTED PATIENTS IN ASELLA REFERRAL AND TEACHING HOSPITAL

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School of graduate studies

This is to certify that the thesis prepared by Chaltu Assefa, entitled nasal colonization of Metacillin-resistant *Staphylococus aurues* among surgical ward admitted patients in Asella Referral and Teaching Hospital 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.

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Table of Contents

List of Tables *page no*

List of abbreviation

AAU: Addis Ababa University

AST: Antibiotics Susceptibility Test

CLSI: Clinical and Laboratory Standards Institute

CA-MRSA: Community Acquired- Methicillin-Resistant Staphylococcus aureus

HC-MRSA: Health Care associated-Methicillin-Resistant Staphylococcus aureus

LA- MRSA: livestock-associated -Methicillin - Resistant staphylococcus aureus

MDR : Multi-Drug Resistance

MRSA: Methicillin-Resistant Staphylococcus aureus

MSA : Manitol Salt Agar

MSSA: Methicillin-Susceptible Staphylococcus aureus

S.aureus: Staphylococcus aureus

SOPs: Standard operating procedures

SCCmec: Staphylococcal Casset Chromosome mec

SSTIs: soft tissue infections

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization occurs when the bacteria are present on the skin without causing symptoms, an immune response, or noticeable cellular damage. However, colonization increases the risk of developing an infection and serves as a significant source of person-to-person transmission. MRSA can be acquired through direct contact with an infected individual, sharing personal items with someone carrying the bacteria, or touching contaminated surfaces or objects.

Objectives: To determine the nasal colonization rate of methicillin-resistant *Staphylococcus aureus* among patients admitted to surgical ward at Assela referral and teaching hospital

Methods: A hospital based cross sectional study design was conducted from June, to November 2024. A total of 283 Nasal swabs were collected during admission and at discharge time and processed using mannitol salt agar and blood agar. Disk diffusion method was used for antimicrobial susceptibility testing. Association of risk factors with colonization of *S.aureus* /MRSA was assessed using Bivariate and multivariate logistic regression. A P value less than 0.05 was taken as statistically significant. All data entry and statistical analysis were done using Statistical Package for Social Sciences (SPSS) version 26 software.

Results: Prevalence of *S. aureus* and MRSA in this study were 16.3 % (46/283) and 7.1% (20/283) respectively. Rate of MRSA among *S. aureus* was 43.4 % (20 /46). We have further evaluated whether the isolated strains have persistently colonized the nasal area,From a total of 283 patients, only 5 patients have persistent MRSA. In this study None of the MRSA isolates were sensitive to penicillin. However, low resistance was observed for sulfamethoxazole trimethoprim and erythromycin.

Conclusion: This study showed lower rate of MRSA colonization among surgical ward admitted patients compared to other study conducted in Ethiopia. Isolates that are resistant to other tested antibiotics including clindamycin are also reported. Therefore,

routine screening and infection preventive practice are crucial in reducing MRSA colonization and its association risks.

Key Words: MRSA, Ethiopia

INTRODUCTION

1.1 BACKGROUND

Staphylococcus aureus was first discovered in 1880 in Aberdeen, Scotland, by surgeon Alexander Ogston from patients with ulcerated sores. *S. aureus* is a gram-positive, non-spore forming, non-motile, coagulase positive bacterium of the firmicutes phylum. *S.aureus* often present on the parts of the human body such as skin, skin glands and mucous membranes as a commensal It is also colonize anterior nares of asymptomatic carriers, who may unknowingly transmit the pathogen within the community or with healthcare facilities. (1,2)

S. aureus has outstanding ability to acquire resistance to antibiotics. Benzyl penicillin was no longer effective for treatment of most *S. aureus* infections within 10 years after its introduction for use because of the acquisition of plasmid-encoded β-lactamase. Penicillin resistant *S. aureus* became pandemic throughout the late 1950s and early 1960s. Methicillin is β-lactam antibiotic invented to treat Penicillin resistant *S. aureus*. However, methicillin resistance *S. aureus* (MRSA) was reported 2 years after the antibiotic was introduced. (3)

Any strain of *S.aureus* that has developed resistantance to β- lactam antibiotics are

Methicillin-resistant staphylococcus (MRSA). In 1961 the first Methicillin-resistant *S.aureus* detected in united kingdom.Methicillin inhibits the penicillin binding proteins (PBPs) including the critical PBP2, which are all involved in the synthesis of the cell wall. In the presence of methicillin, MRSA strains began to express their methicillin-tolerant PBP'2, which compensates for the inhibited enzyme. PBP'2 is encoded by the mecA gene carried by a highly mobile genetic element SCCmec (Staphylococcal Casset Chromosome mec), which is disseminated among strains with apparent ease (4)

S. aureus infections depend on the bacterial growth and expansion in host cells and tissue, the production of surface proteins that start the bacterial adherence to host tissues, the release of extracellular toxins and enzymes that kill host cells and tissues, and the avoidance or inactivation of the host immune system. Among the enzymes that *S. aureus* can produce to increase its pathogenicity and spread throughout the host are coagulase, hyaluronidase, deoxyribonuclease, and lipase. Additionally, it has been determined that extracellular protein toxins that increase pathogenicity include hemolysins, exfoliative toxins (ETs), enterotoxins, toxic shock syndrome toxin 1 (TSST-1), hemolysins, and epidermal cell differentiation inhibitors (EDINs). some of these toxins were found more often in MRSA infections than in non -MRSA ones.(5,6,7)

Based on origin there are three types of MRSA ,Methicillin resistant *Staphylococcus* that are found mainly in hospital patients and long term care facility residents are called health care-associated MRSA (HA MRSA) and MRSA that are found in those who have not had contact with health care facilities are community-associated MRSA (CA MRSA). Infections that have emerged from livestock are called livestock-associated (LA) MRSA (8).

Infections caused by methicillin-resistant strains of *Staphylococcus* aureus are more virulent than infections caused by methicillin-susceptible strains. We can get

MRSA on our skin by touching someone who has the bacteria, sharing things or touching a surface or object that has MRSA on it. Transmission of MRSA by temporary colonization on the hands of healthcare workers is the primary mechanism of MRSA spread in hospitals.(9,10).

MRSA colonization is the presence of bacteria on the skin without the person showing any signs and symptoms or that do not cause a detectable host immune response, cellular damage, have an increased risk of subsequent infection and are an important source of person-to-person transmission. Nasal colonization with *S. aureus* is a vibrant process; a number of factors being responsible for the gain and loss of carriage. Some risk factors for MRSA colonization and infection are previous hospitalization , prolonged hospitalization ,advanced age ,chronic medical illness, implantation of prosthetic devices, Prior and prolonged antibiotic treatment and also Exposure to colonized or infected patient. Recent guidelines published by the Society for Healthcare Epidemiology of America (SHEA) recommend surveillance cultures at the time of hospital admission for patients at high risk for MRSA carriage (9,11)

Generally, carriers were categorized into two main categories as transient or intermittent carriers and persistent or long-term carriers. These categories were explained by the duration of the time period at which a carrier is colonized and by the number and percentage of swabs that are positive for *S. aureus* at different sampling times during the study The fundamental causes of intermittent and persistent carriage are believed to different, despite the fact that the causes are yet unknown. While intermittent carriers may carry multiple strains of *S. aureus* throughout time, persistent carriers are frequently colonized by a single strain over extended periods of time. Additionally, persistent carriers have a greater load of S aureus, which raises the risk of infection and increases dispersal.*S.aureus* loading

and dispersal are higher in nasal carriers who are also perineal carriers.(12,13)

1.2 STATEMENT OF THE PROBLEM

According to World Health Organization (WHO) MRSA has been a substantial and grave danger on the virulent bacteria target test MRSA alone was responsible for more than 100,000 deaths in 3·5 million disability-adjusted life-years were attributed to MRSA globally 2019. It has been reported that those with MRSA infection had a 64% higher

mortality rate compared to those with antibiotics susceptible infection (4).

MRSA is a major cause of healthcare and community acquired infections it causes a wide range of infections including bacteremia, pneumonia, meningitis, endocarditis, skin and soft tissue, surgical site, urinary tract, bone and joint infection and toxic shock syndrome. Infection caused by MRSA is associated with significant morbidity, mortality and costs. (15)

Methicillin resistance is not restricted to the acute care setting. It also has significant effects on costs and charges, the financial burden of MRSA is high due to prolonged hospital stays, increased use of antibiotics and additional care required for infected patients MRSA was associated with 1.2- fold higher median hospital charges compared with MSSA .(16)

Regarding nasal carriage of *S. aureus*, about 20% of the population are persistently colonized with one strain, about 60% are intermittent carriers of varying strains, and the rest of the population never exhibit nasal colonization and A study shows approximately 20% of healthy adults are chronic carriers of *S. aureus*, 30% are intermittent carriers, and 50% are immune for unexplained reasons. Hospital-acquired MRSA infections generally arise from persistent carriers undergoing antibiotic therapy or from intermittent carriers. Both intermittent and persistent MRSA nasal colonization significantly increase the risk of developing an MRSA-invasive infection. In a 2-year period, 21% of persistent and 13% of intermittent carriers developed an invasive infection. (17,18)

The data regarding methicillin-resistant Staphylococcus aureus (MRSA) incidence, obtained from 85 (44%) of the WHO member states, reported values exceeding 20% in all WHO regions, and even 80% in some countries. Interestingly, data from 2013 to 2016 have shown a decrease in the prevalence of MRSA in the United States and Europe, although the mortality rates are still very high. For most European countries, MRSA prevalence among invasive *S. aureus* isolates ranges between 1.2 and 50.5%, with

higher percentages in the Mediterranean countries than in Northern Europe. However, the prevalence of methicillin resistance has increased in some countries (e.g., Spain), reaching values of 25.8%. To make the situation even worse, resistance to other antibiotics (macrolides, lincosamides, and type B streptogramins) and intermediate resistance to vancomycin are currently emerging .(19)

According to WHO Prevalence of MRSA in developed and developing countries varies, due to the strong controlling measures were taken in most developed countries the prevalence of MRSA has been decreasing For instance, MRSA incidence was declined in Europe, United States and Canada over the past eight years while in developing countries like some Africa countries increasingly reported, although rate is still below 50%.(20)

Metacillin resistant *staphylococcus aureus* and also nasal colonization with *staphylococcus aureus* is one of the risk factor for developing infection, particularly in surgical patients. Unidentified MRSA carriers serve as a potential reservoir for transmission. Patients with a history of MRSA colonization in the past 6 months were 8 times more likely to be colonized with MRSA (21).

Researchs done on MRSA colonization among surgical ward admitted patients are limited Therefore ,this study will determine the prevalence of MRSA colonization among surgical ward admitted patients and identifies factors associated with MRSA colonization and antimicrobial susceptibility testing on *S.aureus* and MRSA isolate at Assela referral and teaching hospital as MRSA becoming more prevalent in Ethiopia.

Significance of the study

This study allows us to know the burden of the problem in this particular group of patients. The Study will help, in identifying patients with MRSA colonization in addition; this study helps us to identify persistent colonization of MRSA. This will result in a cost-effective therapy for the patients and reduced financial burden of hospitalization

2 Literature review

A study conducted by John.A et.al in Atlanta,Georgia on 974 Adults presenting for hospital admissionS. aureus was isolated from 205 (21%) of the patients for whom cultures were performed. Methicillin-sensitive S. aureus was isolated from 179 (18.4%) of the patients, and MRSA was isolated from 26 (2.7%) of the patients. All 26 MRSA colonized patients had been admitted to a healthcare facility inthe preceding year, had at least one chronic illness, or both. In multivariate analyses comparing MRSA-colonized patients with control-patients, admission to a nursing home (odds ratio [OR], 16.5; 95% confidence interval [CI95], 1.4 to 192.1; P = .025) or a hospitalization of 5 days or longer during the preceding year (OR, 3.91; CI95, 1.1 to 13.9; P = .035) were independent predictors of MRSA colonization(22).

A prospective observational study conducted by Kepler.A et.al in Texas, shows from 758 patients who had cultures of nares samples performed at admission, 3.4% were colonized with MRSA, and 21% were colonized with MSSA. A total of 19% of patients with MRSA colonization at admission and 25% who acquired MRSA colonization during hospitalization developed infection with MRSA, compared with 1.5% and 2.0% of patients colonized with MSSA (P < .01) and uncolonized (P < .01), respectively, at admission. MRSA colonization at admission increased the risk of subsequent MRSA infection, compared with MSSA colonization (relative risk [RR], 13; 95% confidence interval [CI], 2.7–64) or no staphylococcal colonization (RR, 9.5; 95% CI, 3.6–25) at admission. Acquisition of MRSA colonization also increased the risk for subsequent MRSA infection, compared with no acquisition (RR, 12; 95% CI, 4.0–38) (23).

A systematic literature review and meta-analysis conducted by James A Mckinnell et.al reviewed 4,381 abstracts; 29 articles met inclusion criteria (n = 76,913 patients). MRSA colonization at hospital admission was associated with recent prior hospitalization (odds ratio [OR], 2.4 [95% confidence interval (CI), 1.3–4.7]; P<.01), nursing home exposure (OR, 3.8 [95% CI, 2.3–6.3]; P< .01), and history of exposure to healthcare-associated pathogens (MRSA carriage: OR, 8.0 [95% CI, 4.2–15.1]; Select comorbidities

were associated with MRSA colonization (congestive heart failure, diabetes, pulmonary disease, immunosuppression, and renal failure; P< .01 for all), while others were not (human immunodeficiency virus, cirrhosis, and malignancy). ICU admission was not associated with an increased risk of MRSA colonization (OR, 1.1 [95% CI, 0.6–1.8]; P = .87) (24).

a retrospective cohort study conducted by Kalpana Gupta et al which included all patients admitted to 5 acute care hospitals in England who had nasal MRSA PCR testing within 48 hours of admission and repeat testing within 30 days. Follow-up was up to two years and included acute, long-term, and outpatient care visits. Among 18,038 patients, 91.1%, 4.4%, and 4.6% were never, intermittently, or always colonized at the 30-day baseline. Compared to non-colonized patients, those who were persistently colonized had an increased risk of death (HR 2.58; 95% CI 2.18;3.05) and MRSA infection (HR 10.89; 95% CI 8.6;13.7). Being in the non-colonized group at 30 days had a predictive value of 87% for being non-colonized at 1 year. Conversion to MRSA colonized at 6 months occurred in 11.8% of initially non-colonized patients. Age >70 years, long-term care, antibiotic exposure, and diabetes identified >95% of converters(25).

A review carried out in Asia found an increased numbers of MRSA outbreaks. This systematic review was conducted on the studies published between 2000 and 2016 on MRSA. According to this study more than 75 % of the reviewed papers reports MRSA infection.the most prevalent and resistant proportions between 2000 and 2016 were in mainland china ,Taiwan, Australia, South Korea, Japan, Hong Kong and Thailand. Overall MRSA infection rates in the 19 facilities from year of 2000 to year 2016 ranged from 0 % to 98.4%.the prevalence and resistant rates of MRSA carriers ranged from 0 % to 39 % and 0 % to 88.9 %, respectively (26).

Another systemic review conducted in Asia by Y.C Huang and C.J.chen shows most hospitals in Asia are endemic for multidrug-resistant ,methicillin-resistant *S.aureus*

(MRSA), with an estimated proportion from 28 % (in Hong Kong and Indonesia) to >70% (in Korea) among all clinical *S. aureus* isolates in the early 2010s.(27)

A study conducted in Saudi Arebia on Methicillin-resistant Staphylococcus aureus nasal carriage among patients admitted in hospital shows Of the 220 patients, 90 (40.91%) were found to be nasal carriers of S. aureus. Among these 90 S. aureus isolates, 48 (21.82%) were MRSA. A high prevalence of multidrug-resistant MRSA nasal carriage was found (28).

Based on systematic review conducted by Ephrem et.al, globally, the estimated pooled prevalence of nasopharyngeal carriage of S. aureus using the random effects model was 22%. The highest rate of nasopharyngeal carriage of S. aureus observed in Europe 25%, followed by studies in Asia and Africa which was 22% and 21%, respectively. On the other hand, the highest nasopharyngeal carriage of S. aureus was observed in the children with age range of 6-15 years that accounted 25%. The estimated pooled global nasopharyngeal carriage of methicillin resistant S. aureus (MERSA) was 13%, while the nasopharyngeal carriage of methicillin sensitive S. Aureus (MSSA) was 81%.(29)

A cohort study done in Netherland by Kalmeijer MD, a total of 272 patients were enrolled out of these 6.6% experienced SSI, both the superficial and deep SSI in different individual and These infections led to serious cases and caused 286 extra days in hospital, this study shows as high –level nasal carriage of S*.aureus* was the most important and risk factor for developing SSI (30).

MRSA and nasal colonization with Staphylococcus aureus is one of the risk factor for developing infection. Approximately 10% to 20% of MRSA colonization and or develop infection occur during hospitalization-.a retrospective cohort study conducted in California teaching hospital of all adults with MRSA-positive shows patients with high MRSA nasal burden incurred increased risks of invasive disease, even after accounting for host factors for infection, another retrospective study conducted in US Atlanta VA medical center suggests defining MRSA colonization as a risk factor for infection and also in this study the death rate among MRSA colonized patients was considerably greater than those without nasal colonization (53% vs. 34%, respectively) (31).

A study carried out in Africa by Matthew E. F et al in January 2013 on MRSA in Africa: They sought to assess the prevalence of methicillin-resistance among *S.aureus* isolates in Africa. They included articles published in 2005 or later reporting for the prevalence of MRSA among *S.aureus* clinical isolates. Thirty-two studies were included. In Tunisia, the prevalence of MRSA increased from 16% to 41% in between 2002–2007, while in Libya it was 31% in 2007. In South Africa (S.Africa), the prevalence decreased from 36% in 2006 to 24% during 2007–2011. In Botswana, the prevalence varied from 23–44% in years 2000–2007. In Algeria and Egypt, the prevalence was 45% and 52% from 2003–2005, respectively. In Nigeria, the prevalence was higher in the northern part than the southern part. In Ethiopia and Ivory Coast, the prevalence was 55% and 39%, respectively. The prevalence of MRSA was lower than 50% in most of the African countries, although it appears to have risen since 2000 in many African countries, except for South Africa (32).

A cross-sectional study conducted by Ifunanya P et al on the prevalence and multidrug -resistant (MDR) pattern of Methicillin-resistant S. aureuscarriage among surgical patients, patient relatives and healthcare workers (HCW) in a tertiary health facility in Uyo-Nigeria shows that Overall, S. aureus and MRSA carriage rates among the participants were 102 (51.0%) and 22 (11.0%), respectively. Population-specific carriage rates of S. aureus and MRSA among surgical patients (n=65) were 41 (63.1%) and 15 (23.1%); patient relatives (n=65), 22 (33.8%) and 4 (6.2%), while HCW (n=70) were 39 (55.7%) and 3 (4.7%), respectively(33).

A study conducted by Naghavi-Behzad et al. On patients who were confined to bed in the surgery ward, the authors assessed if patients had MRSA infection when hospitalized and once when they were discharged. In this study a total of 475 patients were included, out of all 108 patients (22.8%) had *S.aureus*. Among collected *S.aureus* colonies, erythromycin resistance, was the most frequent antibiotic resistance, also resistance to vancomycin was 0.4% that was the least. (34). Similar study was conducted by Deboye D O, Kolawole.A et.al in Nigeria patients hospitalized in the surgical wards Among 192 persons tested, the overall prevalence of *S. aureus* carriage

was 31.8% of these isolates, 11.5% were methicillin-resistant (MRSA) (35). In other a cross-sectional study was conducted in Dar Es Selam Tanzania by Alfred et.al on Methicillin resistant Staphyloccous aureues colonization on hospital admitted patients of the 169 patients the prevalence of MRSA was 11.8 % . All MRSA isolates were highly resistant to penicillin and erythromycin, and 17 (85.7%) were highly sensitive to vancomycin (36)

A cross sectional study was conducted by Tekalign KejelaA and Fili Dekosa among patients admitted to Mettu Karl Referral Hospital. A total of 384 patients admitted to medical, pediatric and surgical ward, wound and nasal swabs collected from inpatients. A total of 126 staphylococcus aureus was isolated from 384 patients from those 126 Staphylococcus aureus isolated, 57.1% (72) were MRSA and 7.9% (10) were VRSA. Of the 166 samples collected from patients in the surgical ward, the rates of isolation of MRSA and VRSA were 21.1% (35/166) and 4.8% (8/166), respectively. A high rate of isolation of MRSA and VRSA was recorded among patients admitted to surgical wards compared with medical and paediatric wards (37).

A meta-analysis conducted by Alemayehu, Abeba & Asnakew on nasal colonization of MRSA ,A total of ten studies with 2495 nasal swab samples were included in this meta-analysis, and the overall pooled estimated prevalence of *S. aureus* and MRSA nasal colonization in Ethiopia were 30.90% [95% CI 21.81–39.99%], 10.94% [95% CI 8.13–13.75%] respectively. Subgroup analysis was also noted in different regions of Ethiopia, henceforth Oromia region ranked first 21.28% [95% CI 8.22–34.35%], followed by Amhara region 6.78% [95% CI 3.02–10.54%], whereas relatively low magnitude of MRSA colonization was demonstrated from Tigray region 4.82% [95% CI 2.18–7.45%]. (38)

This cross-sectional study was conducted by Barena and Fetene on nasal carriage of methicillin resistant staphylococcus aureus strains among inpatients of Jimma hospital, south western Ethiopia, out of the total 152 inpatients enrolled in the study, one hundred forty-four swabs cultured on mannitol salt agar grew Staphylococcus species out of which 85(59%) were coagulase positive and 59 (41%) were coagulase negative. Antibiotic sensitivities of 85 Staphylococcus aureus isolates to ampicillin,

chloramphenicol, trimethoprim-sulphamethoxazole, oxacillin, erythromycin, kanamycin, gentamycin and clindamycin showed resistance pattern of 87.1%, 70.6%, 68.2%, 51.8%, 42.4%, 16.5, 15.3% and 12.9% respectively. 51.8% of the isolates were methicillin resistant Staphylococcus aureus and 72.9% were multi resistant (39).

According to meta-analysis study in Ethiopia by Fentahun Tarekegn et al on Methicillin resistant Staphylococcus aureus the authors included a total of 20 studies and showed that the pooled prevalence of methicillin resistant Staphylococcus aureus was 32.5% (95% CI, 24.1 to 40.9%). Moreover, methicillin resistant Staphylococcus aureus strains were found to be highly resistant to penicillin, ampicillin, erythromycin, and amoxicillin, with a pooled resistance ratio of 99.1, 98.1, 97.2 and 97.1%, respectively (40).

3. Objectives

3.1 General objective

- To determine colonization of methicillin-resistant Staphylococcus aureus among patients admitted to surgical ward at Asella referral and teaching hospital

3.2 Specific objectives

- To determine prevalence of methicillin-resistant Staphylococcus aureus among patients admitted to surgical ward

- To identify factors associated with methicillin-resistant Staphylococcus aureus
- To determine the antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus isolates.
- To determine persistent Methicillin - resistant *S.aureus* colonization among patients admitted to surgical ward

4. Materials and Methods

4.1 Study area

The study was conducted at Asella referral and teaching hospital, ATRH is located in Asella town, which is found in the Arsi Zone of the Oromia Region at about 175 km from Addis Ababa, the country's capital. The hospital provides health services to about 3.5 million populations in Arsi and the nearby zones. Among these, about 3000-4000 operations are performed annually. The hospital has 321 beds, 54 beds for the surgical ward, with more than 5 departments

4.2 Study Design and period

A prospective hospital-based cross sectional study was conducted from June 2024 to November 2024

4.3 Population

4.3.1 Source Population

All patients admitted to Asella referral and teaching hospital during the study period

4.3.2 Study Population

All patients admitted to surgical ward and who fullfield the inclusion criteria was included.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion criteria

All patients admitted to surgical ward during the study period

4.4.2 Exclusion criteria

All patients those did not consent to participate in the study were excluded.

5.5 Study variables

5.5.1 Dependent variable

- Prevalence of Methicillin resistance *Staphylococcus aureus*
- Antimicrobial sensitivity patterns of the *S. aureus*.
- Persistent MRSA

5.5.2 Independent variable

- age,
- sex,
- hospitalization

- Presence of indwelling medical device
- Oral antibiotic usage
- Presence of chronic wound
- Chronic disease

5.6 Sample size calculation and Sampling method

5.6.1 Sample size calculation

The sample size was calculated using single proportion formula by taking the prevalence of MRSA from mettu,Ethiopia, which was 21.1%(37).

Total study subjects: n = z

$$\frac{2\,p\,(1-p)}{d2} = 283$$

Where:

n= sample size

Z= Standard normal deviate at 95% confidence interval

P= Proportion of target population infected with MRSA taken 21.1%from previous similar study in Ethiopia mettu karl hospital.

q= 1-p

d= degree of freedom.

Z= 1.96, p=21.1% q=78.9% d=0.05

Thus;

$n = 1.96^2 \times 0.211 \times 0.789 / 0.05^2$

=255

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

n= 224/0.9= 283

5.5.3 Sampling technique

Convenience sampling techniques was applied to recruit the study participants until the achievement of the expected sample size within the given study period.

5.6 Measurement and Data collection

5.6.1 Data collection procedure

After providing informed consent clinical information was collected from each participant. Each patient was screened at the first date of admission and before leaving the center. In addition the potential association factors for MRSA colonization was collected from each participant.

5.6.2 Laboratory Methods

5.6.2.1 Nasal swabs collection

Sterile cotton swabs were used to collect swabs from the anterior nares of the participants selected. Specimens for culture was obtained by firmly rotating a new pre moistened cotton-tipped swab against the anterior nasal mucosa for 3 second in each anterior nares with the same swab and taking sample at two time points (before and after surgery) ,label the swab, and transport specimen to the laboratory(41).

5.6.2.2 Isolation and Identification

After the sample has been collected aseptically, all samples were inoculated on both Blood Agar plate and mannitol salt agar (MSA) and incubated at 37ºC for 24-48 hours. Bacterial colonies showing typical characteristics of *S. aureus* (i.e., beta hemolytic on blood agar and colonies with golden yellow pigmentation on mannitol salt agar) was subjected to subculture, for those having growth on the subculture media, by isolating the pure colony gram stain and biochemical tests were performed. Biochemical tests, catalase and coagulase were performed. Those Catalase, coagulase and gram positive bacteria appearing in grape like cluster were considered as *Staphylococcus aurues (*42).

5.6.2.3 Antimicrobial susceptibility test

Antimicrobial susceptibility testing's was performed for isolated organisms on Kirby-Bauer's disk diffusion on Muller Hinton agar according to Clinical and Laboratory Standards Institute guideline (CLSI 2018) (43).The antibiotic susceptibility testing was performed on the followsing antibiotic discs; Cefoxitin (CXT, 30µg), Clindamycin (DA,2µg), Erythromycin (ERY,15µg) Trimethoprim Sulphamethoxazole (SXT,1.25µg/23.75µg), Penicillin (PG, 10units) Ciprofloxacillin (CIP,5µg) andGentamicin (GEN, 10µg). After 18-24 hours incubation at 37ºC, zone of inhibition was measured and reported as susceptible (S) and resistance (R) based on the clinical and laboratory standard institute (CLSI) guidelines.

Detection of MRSA strain by cefoxitin disc diffusion methods

The isolate *S.aureus* was screened for methicillin resistance following the clinical laboratory standard institute (CLSI, 2018) modified kirbauer disc diffusion method. An overnight culture from sheep blood agar was plated on Muller-Hinton agar and cefoxin (30) disk was placed on the inoculated plate. Zone diameter was measured using a ruler and a zone size of >22 mm after 24 hour incubation at 37oc was considered as susceptible for cefoxitin while, those isolate of *S.aureus* with an inhibition size < 21mm was considered as resistant to cefoxitin and defined as MRSA.

5.7 Data Quality Assurance

Data quality was ensure through use of standardized data collection materials, pretesting of the questionnaires, proper training before the start of data collection and intensive supervision during data collection by the principal investigator. For laboratory analysis pre-analytical, analytical and post-analytical stages of quality assurances that are incorporated in standard operating procedures (SOPs) of the microbiology laboratory of Addis Ababa public health research and emergency management core process was strictly followed. In addition, well-trained and experienced laboratory professionals have participate in the laboratory analysis procedure.

5.7.1. Pre-analytical phase

The nasal swab collection container were labeled and dated. The specimen was collected, transported as quickly as possible. All patient information collected during the study period was checked for its clarity and completeness in a regular basis. Each lot of the medium was checked for expiration dates prior of quality control

5.7.2 Analytical phase

All materials, equipment and procedures adequately controlled. Quality control of culture media was done for sterility test and the ability to grow the control bacteria strains. To standardize the inoculum density of bacterial suspension for the susceptibility test, a barium sulfate (BaSO4) turbidity standard, equivalent to a 0.5 McFarland standard was used and standard strain of S. *aureus* (ATCC-25923) was used as Control bacteria strains for both media and antibiotics discs. Standard operating procedure (SOPs) of the microbiology laboratory of Addis Ababa public health research and emergency management core process strictly followed and the results was checked by the senior microbiologist.

5.7.3 Post-analytical phase

The results recorded with the patients' identification number. In order to avoid the errors in the results of the test, the reporting was repeatedly checked and evaluated by the head of the department before the results given to the caregiver. Appropriate action(s) was taken when a result has serious patient or public health implication.

5.8 Data Processing and Analyses

All data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 27 software. Bivariate and multivariate logistic regressions were used to

determine the association between dependent and independent variables. Finally the results were presented in words, tables and graphs. P value less than 0.05 was considered as statistically significant.

5.9. Ethical consideration

This research project was approved by the Department of Medical Laboratory Sciences, CHS, AAU. To conduct the study, permission was obtained from Asella referral and teaching hospital. Study subjects recruited after they become informed about the objectives and use of the study and then after they gave informed consent. Minimal risk associated with the process of sampling; it was the same as taking specimen for culture and sensitivity in the routine laboratory. For all confirmed MRSA the responsible clinician of the study subjects informed. All the information contained within the study was confidential.

5.10 Dissemination and public engagement

The result of this study was disseminated to Department of Medical Laboratory Science, Addis Abeba University and Assela referral and teaching hospital. It will also be disseminated through presentation on local or scientific conference; it would be published on journal.

5.11 Operational definitions

Methicillin-resistant *Staphylococcus aureus*: Is defined as the strains of *S. aureus* that are resistant to cefoxitin ((30 µg) using disk diffusion method on Mueller Hinton agar.

Multi Drug Resistance: - bacterial resistance for three or more antibiotics

Persistent Methicillin-resistant *Staphylococcus aureus*: - a condition where MRSA bacteria remain in the body for at least five days

6. Result

6.1 Socio demographic characteristics

A total of 283 Patients were included in the study. The age of study participants ranged from 12 - 90 years with mean age of 40.05 ± 16.918 years. Out of these, 130 (45.9%) were females and 153 (54.1%) were males. In terms of age distribution, the majority fell within the 19–29 yrs (25.8%) and 30–40 yrs (24.7%) age groups. The youngest age group, under 18 years, had the smallest representation at 7.1%. This distribution suggests a relatively balanced gender representation and a concentration of participants in young to middle adulthood. (Table1).

Table 1 socio demographic characteristics of the study participants for nasal colonization in Asella teaching and referral hospital

Variables	Category	Frequency	Percent
Gender	Female	130	45.9%
	Male	153	54.1%
Age	<18	20	7.1%
	19-29	73	25.8%
	30-40	70	24.7%
	41-51	48	17.0%

	52-64	41	14.5%
	>65	31	11%

6.2 Prevalence of Nasal *S.aureus* colonization and MRSA colonization

6.2.1 Prevalence of Nasal *S.aureus* and MRSA colonization

The prevalence of *S. aureus* and MRSA in this study was 46 (16.3%) and 20 (7.1) % respectively. MRSA accounted for 43.4 % of 46 *S.aureus* isolates. Of the total 20(7.1%) MRSA colonization, male comprised 12(60.0%) while female comprised 8(40%). Like MRSA colonization, there was high colonization rate of *S.aureus* among male 27(58.7%) when compared with female 19(48.3%). Rate of colonization was slightly higher among age group 19-29 of the participants (Table 2).

6.2.2.Persistent MRSA infection

We have further evaluated whether the isolated strains have persistently colonized the nasal area. Accordingly, persistent MRSA was assessed from surgical ward admitted patients by taking sample at two time points (before and after surgery). From a total of 283 patients, 8 patients (17.4 %) had persistent Staphylococcus aureus (S. aureus) colonization while only 5 patients have persistent MRSA. Of the five patients, two were female and three were male (Table 3)

Table 2: Age and sex distribution of *S.aureus* and MRSA among surgical ward admitted patients in asella teaching and referral hospital

Study characteristics	Category	*S.aureus colonization*	MRSA colonization	Negative	P- Value
Gender	Female	19(41.3)	8(40.0%)	103(47.5%)	

	Male	27(58.7%)	12(60.0%)	114(52.5%)	0.98
Age	<18	4(8.7%)	1(5.0%)	15(6.9%)	0.217
	19-29	12(26.1%)	5(25.0%)	54(24.9%)	0.191
	30-40	8(17.4%	4(20.0%)	53(24.4%)	0.318
	41-51	9(19.6%)	2(10.0%)	37(17.1%)	0.09
	52-64	8(17.4%)	2(10.0%)	34(15.7%)	0.115
	>65	5(10.9%)	6(30.0%)	5(10.9%)	

Table 3 Persistent MRSA and S. aureus Colonization Among surgical ward admitted patients in Asella teaching and referral hospital

Persistent colonization	Total positive cases	Persistent cases in number (n)	Percent
S.aureus	46	8	17.4%
MRSA	20	5	25%

6.3. Analysis of risk factors for *S.aureus* and MRSA colonization

None of the socio-demographic characteristics mentioned in this study were found to be associated with *S.aureus* and MRSA colonization rate (Table 2). By bivariate Analysis, *S.aureus* colonization rate was statistically significant in individuals with the history of hospitalization (OR = 2.648; 95% CI: 1.32–5.28; p-value = 0.029) and presence of indwelling medical device (OR = 2.243; 95% CI: 1.083–4.632; p-value = 0.029) (Table 3). In bivariate logistic regression analysis there were no significant associations between MRSA colonization and any of the independent variables (Table 4).

Table 4 bivariate Analysis of risk factors for Nasal colonization of *S.aureus* and MRSA among surgical ward admitted patients in Asella teaching and referral hospital

Variable	category	Frequency	*S.aureus* colonization			MRSA colonization		
			OR	OR(95%CI)	P-value	OR	95%CI	P-value
Hospitalization	Yes No	159 124	2.748	(1.327-5.282)	0.006	2.801	0.990-7.931	0.05
Presence of indwelling medical device	Yes No	185 95	2.243	(1.083-4.632)	0.029	1.502	0.559-4.034	0.420
Oral antibiotic usage	Yes No	93 190	0.762	(0.570-2.155)	0.762	1.108	0.427-2.878	0.820

Presence of chronic wound	Yes No	34 249	0.794	(0.320-2.393)	0.794	0.367	0.048-2.832	0.336
Chronic disease	Yes No	60 223	0.930	(0.355-3.411)	0.607	0.930	0.355-3.411	0.607

6.4 AST of the isolated *S. aureus*

All the isolates of *S.aureus* were tested for susceptibility to selected antibiotics. Antimicrobials used in this study were, Penicillin, Cefoxitin, Erythromycin, Clindamycin, Trimethoprim - Sulphamethoxazol, Ciprofloxacillin, and Gentamicin. The present study has demonstrated the existence of different levels of resistance of *S.aureus* to commonly used antimicrobial agents in the study area. Significant proportions of S.aureus isolates (91.3%) were susceptible to Erythromycin, Trimethoprim - Sulphamethoxazol (78.3%), Clindamycin (87.0%), Gentamicin (67.4%). Gentamicin,Trimethoprim–Sulphamethoxazol, Clindamycin and Ciprofloxacillin (6.5%), (4.3%) (2.2%) and (6.5%) were intermediate each respectively. All isolates were resistant to penicillin as shown in (Table 5).

Additionally, 43.5% (n=20) of the *S.aureus* isolates were found to be resistant to Cefoxtin, which is a surrogate for the prevalence of MRSA. All 20(100%) MRSA isolates were resistant to penicillin and sensitive to clindamycin. The susceptibility profile of Erythromycin, Gentamicin, Ciprofloxacillin and Trimethoprim – Sulphamethoxazol of MRSA isolates were 90%, 60%, 65%, 35% and 100% respectively (Table 5).

Table 5 Antimicrobial Susceptibility pattern of *S.aureus* and MRSA by Disk diffusion

	S.aures			MRSA	
	Resistant	Sensitivie	Intermidiate	Resistant	Sensitivie
Gentamicin	26.1%	67.4%	6.5%	40.0%	60.0%
sulfamethoxazole trimethoprim	17.4%	78.3%	4.3%	15.0%	85.0%
Erythromycine	8.7%	91.3%	0.0%	10.0%	90.0%

Clindamycin	10.9%	87.0%	2.2%	100.0%	0.0%
Ciprofloxacillin	30.4%	63.0%	6.5%	35.0%	65.0%
Penicillin	100.0%	0.0%	0.0%	100.0%	0.0%
Cefatoxin	43.5%	56.5%	0.0%	100.0%	0.0%

6.5 Persistent MRSA colonization and drug resistance

We further tried to compare the antibiotic susceptibility pattern of persistent methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from first and second swabs. The results indicate that MRSA exhibited high resistance to most tested antibiotics, including erythromycin, clindamycin, penicillin, and cefatoxin, with all isolates being resistant in both swabs. Resistance to ciprofloxacillin remained consistent, with only one isolate susceptible. Sulfamethoxazole-trimethoprim and gentamicin showed moderate efficacy, with a slight decrease in susceptibility in the second swab (shown in table 6).

Table 6: Antibiotic susceptibility pattern of persistent MRSA before and after surgery

Isolates number	Gentamicin	Erythromycin	Ciprofloxacillin	Clindamycine	Sulfamethaoxazole trimethoprim	Penicillin	Cefatoxin
1 st MRSA X	S	R	R	R	S	R	R
2 nd MRSA X	S	R	R	R	R	R	R
1 st MRSA Y	S	R	R	R	S	R	R

2 nd MRSA Y	S	R	R	R	S	R	R
1 st MRSA Z	R	R	R	R	S	R	R
2 nd MRSA Z	R	R	R	R	S	R	R
1 st MRSA W	S	R	S	R	R	R	R
2 nd MRSA W	R	R	S	R	R	R	R
1 st MRSA V	R	R	R	R	S	R	R
2 nd MRSA V	R	R	R	R	S	R	R

S = susceptible; R = resistant

7. DISCUSSION

In the present study, the overall prevalence of isolation of *S.aureus* from surgical ward admitted patients was 16.3%. The finding of this study was in line with, a related study done in Chang Gung Medical hospital at Linkou which has demonstrated an isolation rate of 16.4% but higher than a study conducted in sudan, (44) with the rate of isolation of *S.aureus* among surgical patients at kosti teaching Hospital of 11.2%. Similarly, a study done in Hawassa University Comprehensive Specialized Hospital, Ethiopia which reported an isolation rate of 13.6% from nasal swabs of admitted patients (45).

Our rate of *S.aureus* isolation (16.3%) is lower than that of the study conducted in Southwest Ethiopia (46) and Northwest Ethiopia (47) where the isolation rate was 32.8% and 29.3% respectively. The low prevalence of *S.aureus* colonization in our study might be due to difference in rate of patient load, study area, geographical area and study population may contribute to the difference.

In our study prevalence of MRSA was 7.0%, which was comparable to rates reported for Jimma, Ethiopia (8.4%) (48) and India (8.6%) (49), but was higher than the prevalences of MRSA reported in Netherlands (0.13%) (50), Germany (2.1%) (51) and Ireland (2.7%) (52) and also a study done in Indonesia Mangunkusumo Hospital shows a low prevalence of MRSA colonization anterior nasal swab (0.8%) (53). A recent study by Santosaningsih, et al. found that MRSA carriage rate was 4.3% among surgical patients in three academics hospitals in Indonesia. In the study, the screening was performed at the time of discharge and patients that discharged within 48 h of admission were excluded. Subgroup analysis of three hospitals that participated in this study revealed a significant variation number of MRSA carriage (54).

A key finding in our study is that 5 patients exhibited persistent MRSA colonization, indicating a prolonged risk of infection. Persistent MRSA carriers have a significantly higher risk of developing invasive infections, requiring longer hospital stays and contributing to nosocomial transmission (55). Persistent colonization has been linked

to recurrent MRSA infection and increased healthcare costs, highlighting the need for effective screening and decolonization measures (55, 56).

In this study, recent hospitalization was a risk factor for nasal colonization of *S.aureus* . This was also showed by other studies conducted in Mekelle; Ethiopia (57), Iran (58) and Pennsylvania (59). Presence of indwelling medical device within the past year was found to have association by bivariate and multivariate analysis with *S.aureus* colonization. This result was supported by other studies conducted in Mekelle, Northern Ethiopia (57). In the current study, Colonization by MRSA was not associated with any one of the variables. And this finding was consistent with the study done in Northwest Ethiopia (60) where colonization by MRSA was not associated with any one of the variables.

In our study the antimicrobial susceptibility pattern of MRSA isolates showed 100 % resistance to Penicillin which was similar to the study in North east Ethiopia (61). In our study Erythromycin (90%) Gentamicin(60%), Ciprofloxacillin(, 65%,,) and Trimethoprim – Sulphamethoxazol, (35%) were suceptabile of MRSA isolates. A study conducted in Nigeria reported that, 73.3% of MRSA isolates were sensitive to Clindamycin and 33.3%,26.7%, 33.3% and 20% of MRSA isolates were sensitive to Gentamycin, Trimethoprim sulphamethoxazole, Erythromycin and ciprofloxacillin respectively (62).

The observed resistance patterns in our study highlight the growing challenge of managing MRSA infections in clinical settings. The high resistance to penicillin and reduced susceptibility to commonly used antibiotics such as Gentamicin, Ciprofloxacin, and Erythromycin underscore the need for regular antimicrobial resistance surveillance. These findings are consistent with studies from other regions, including Northern India and Debre Markos, which also reported varying resistance levels among MRSA isolates [63, 64]. Such resistance trends may limit treatment options and necessitate the use of more potent or reserved antibiotics, emphasizing the importance of rational antibiotic use and infection control practices in hospital wards

Differences in susceptibility pattern of the isolates could be due to differences in

geographical area and the time where MRSA isolates first occurred (the longer the MRSA are present in a geographic area, the more likely they are to be resistant to other antibiotic classes). MRSA isolates were highly susceptible to most tested antibiotics, these could be due to CA-MRSA strains were capable of resisting only β- lactam antibiotics as the result of carriage of genetic element SCCmec type IV. SCCmec type IV is one of the shorter SCCmec variations less likely to carry multi-drug resistance (65).

9. Limitation of the study

In this study, only two samples were collected from each patient. sampling over multiple time points requires significant resources, including laboratory costs which were beyond the financial capacity of this study This limited sampling frequency may have restricted our ability to capture persistent MRSA. Moreover, the absences of molecular methods to characterize MRSA are significant limitations of the current study. Future studies should incorporate multiple time-point sampling and molecular typing of MRSA strains to better understand transmission dynamics and resistance mechanisms.

10. Conclusion and recommendation

Nasal colonization with methicillin –resistant staphylococcus aureus (MRSA) serves as a significant reservoir for the spread of infection. Individual colonized with MRSA is at an increased risk of developing invasive infection and contributing to the transmission of the pathogen to others.This study showed lower rate of MRSA colonization among surgical ward admitted patients compared to other study conducted in ethiopia.Isolates that are resistant to other tested antibiotics including clindamycin are also reported. The data have important implication for quality of patients care and effective control measures, such as routine screening and strict infection preventive practice are crucial in reducing MRSA colonization and its association risks. I will recommend implementing routine screening in health center especially in high-risk populations

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Annexes

Annex 1: English version of participant information sheet, assent, consent & questionnaire

I. Participant information sheet

Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis

Ababa University, Addis Ababa, Ethiopia

Title of the Research Project: colonization of methicillin-resistant Staphylococcus aureus among patients admitted to surgical ward at St. Paul's Hospital Millennium Medical College.

First of all I would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Background: Any strain of S.aureus that has developed resistantance to β- lactam antibiotics are Methicillin-resistant staphylococcus (MRSA).Infection caused by methicillin-resistant strain of S.aureus is more virulent than infection caused by methicillin- susceptible strain. We can acquire MRSA on our skin by touching someone who has the bacterium, sharing things with someone who has MRSA on the skin and touching surface or objects that have MRSA on them.

Aim of the study

The purpose of this study is to determine colonization of methicillin-resistant Staphylococcus aureus among patients admitted to surgical ward at St. Paul's Hospital Millennium Medical College., Addis Ababa, Ethiopia.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, based on the diagnosis result you will be treated accordingly.Most importantly, the result of the study will be beneficial to design effective prevention and control measure for MRSA. Hence, you are indirectly benefiting other patients and the society in this respect.

Risks and complication

There are no anticipated risks to your participation. As routine laboratory procedure blood sample will be taken once from your peripheral vein. During sample collection you may feel some discomfort but this does not produce serious pain.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. Participants will not be prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The information collected about you will be coded using numbers. No personal information will be disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

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

Chaltu Assefa(PI)

Signature: _____ Date: _____

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

PI Address: Chaltu Assefa: Department of Medical Laboratory Sciences, Collage of

Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: chaltuassefa42@gmail.com Mobile-0913035590

Department of medical laboratory sciences, CHS, AAU, Tel-0112755170

II Consent form

I have been informed about the study which plans to determine colonization of methicillin-resistant Staphylococcus aureus among patients admitted to surgical ward at St. Paul's Hospital Millennium Medical College., Addis Ababa, Ethiopia.

The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care. It is therefore with full understanding of the situation that I agreed to give the informed assent voluntarily to the researcher to give nasal swab for the mentioned study. I agreed that the specimen would be tested for MRSA. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis will be given to the Doctor who follow me and that I may ask the information if I want.

I _____ hereby give my assent for giving of the requested information and nasal swab for this study.

Participant code:_____ Signature: _____ Date: _____

III. Questionnaire

Addis Ababa University Collage of Health Sciences, School of Allied Health Science Department of Medical Laboratory Science.

Questionnaire for the demographic characteristics and associated factors of MRSA among surgical ward admitted patients in SPHMMC

part one. Socio- Demographic Characteristics of the Study participants.

1. Age _____

2. Sex a. Male b.Female

Part two :Patient pre-admission History

1. Date of admission in the hospital dd /mm/yy
2. Is there any history of hospitalization in the past 6 month ? A) yes B) No
3. If the answer is yes for no.1 ,How long days did you stay in the hospital ? ……
4. .Did you used any medical equipment's in your body such as like Catheter, Drug lines. etc. 1. Yes 2. No
5. Do you have taken antibiotic in the last six months? A) YES B) NO
6. Do you have chronic wound? A) YES B) NO
7. Do you have history of surgery recently? A) YES B) NO
8. If your answer for question no.7 is yes ,what was the reason ?
9. Do you have a history antibiotics prescribed in the last 6 months? 1.Yes 2. No
10. If your answer for question no.9 is yes ,what was the prescribed antibiotics?

Part three : post admission

1. How long did you stay in the hospital ? …….
2. Did you have a new infection within the last 48 hours after admission ? A) yes B) NO
3. If your answer yes for question no.2 what was the cause ?
4. Do you have history of blood transfusion A) YES B) NO

Annex 2 oromic version of participant information sheet, assent, consent & questionnaire

I. Waraqaa Odeeffannoo Qoodamaa**

Qabeenya Saayinsii Fayyaa Laaboratorii, Kuullaajjii Saayinsii Fayyaa Walitti Qabame, Yuunivarsiitii Addis Ababa, Addis Ababa, Itoophiyaa

Maqaa Pirootii Qorannoo: Qoqqoodama Staphylococcus aureus Mettiisiliinii Dhabe (MRSA) kan qorannoo keessatti qabamanii mana yaalaa sirummaa Asella referral and teaching hospital.

Akka duraan isin gaafadhu, hiriyoota kana keessatti hirmaachuufi gumaachuuf galata guddaan isiniif haa tahu. Odeeffannoo wal faana qorannoo kana dubbisaa yookiin dubbifamaa dhaggeeffachuun mirkaneeffadhaa. Yoo gaaffi qorannoo irratti qabdan, achumatti gaafadhaa.

Odeeffannoo Duubaalaa

Duubaala:Sanyii Staphylococcus aureus kan mettiisiliinii fi antibiotikii beta-laaktamii dhabe MRSA jedhama. Dhukkubni MRSA fidaan hamma kan mettiisiliinii dhabe hin qabne (MSSA) caalaa hammeessaadha. MRSA qaama keenya irratti akka nutti dhufu namni MRSA qabu qaama isaa nu tuqe, wantoota isa wajjin qooduun, yookiin wantoota MRSA qaban tuqachuun danda’ama.

Kaayyoo Qorannoo

Kaayyoon qorannoo kanaa qoqqoodama MRSA kan qabamanii mana yaalaa sirummaa Asella referral and teaching hospital keessatti madaaluudha.

Faayidaa Qoodamtootaaf

Qoodamtoonni qorannoo kanaa qaaliin maallaqaafi kkf argachuu hin danda'an. Garuu, deebii qorannoo irraa kan argatan irratti hundaa'een, daa'imman keessan fayyaa ta'uu danda'a. Deebiin qorannoo kanaa tajaajila ittisaa fi MRSA balaa dhabamsiisuu keessatti gargaara. Kanaaf, akkaataa armaan gadii namoota biroo fi hawaasa waliin faayidaa argachuu dandeessu.

Riskii fi Rakkoowwan

Hirmaachuun keessan irratti riskii hin jiru. Akka adaama laaboratorii, dhiigni keessan immoo dhangala'aa irraa tokko qofa fudhatama. Yeroo dhangala'aa fudhatu rakkoo xiqqoo ta'uu danda'a, garuu dhukkuba guddaa hin fidu.

Iccitii Guddataa

Odeeffannoo keessan sirriitti eeguuf jecha lakkoofsii kan qoodama. Maqaa keessan dhaabbataa biroo hin himamu. Qoodamtoonni yeroo kamiiyyuu qorannoo irraa dhiisuu danda'u. Deebii laaboratorii lakkoofsii isaanii fayyadamuun argachuu danda'u. Odeeffannoo keessanis lakkoofsii qoodama.

Waadaa Qorataa Guddaa

Ani akkaataa armaan gadii qorannoo kanaa hundaaf deeggarsa saayintifikii, hawaasummaa, fi teeknikaa ta'e kennuuf galata galchadha. Deebii qorannoo hundaafis dhaabbataa hundaaf kenna.

Chaltu Assefa (Qorataa Guddaa)

Mallattoo: _____ Guyyaa: _____

Yaadannoo: Yoo gaaffi qorannoo irratti qabdan, Chaltu Assefa qubatee gaafadhaa: Qabeenya Saayinsii Fayyaa Laaboratorii, Kuullaajjii Saayinsii Fayyaa Walitti Qabame, Yuunivarsiitii Addis Ababa, Addis Ababa, Itoophiyaa.

E-mail: chaltuassefa42@gmail.com Bilbila: 0913035590

Qabeenya Saayinsii Fayyaa Laaboratorii, CHS, AAU, Bilbila: 0112755170

II. Foormii Idileetti

Qorannoon kun qoqqoodama MRSA kan qabamanii mana yaalaa sirummaa St. Paul's Hospital Millennium Medical College, Addis Ababa, Itoophiyaa keessatti madaaluuf kan ta'e nibeekee jira. Kaayyoo fi faayidaa qorannoo kanaas nibeekee jira. Hirmaachuu, qooduu, yookiin yeroo kamiiyyuu irraa dhiisuu kophaa kiyya ture. Hirmaachuun kiyya fayyaa kiyya irratti hin bu'uura. Kanaaf, mirga kiyyaan qorannoo kanaaf swaabaa funyaan kennuuf idileetti gumaacha. Swaabaan kana MRSA madaaluuf fayyadamuu isaa nibeekee jira. Gaaffiin kiyya hundaaf deebii argadhe. Deebiin qorannoo kanaa doktora koo bira geessisuufi yoo barbaadde nibeekuu danda'a.

Ani _____, swaabaa fi odeeffannoo kennuuf idileetti gumaacha.

Lakkoofsi Qoodamaa: _____ Mallattoo: _____ Guyyaa: _____

Gaaffilee

Yuunivarsiitiin Addis Ababa, Koollejjii Saayinsii Fayyaa, Mana Barumsaa Saayinsii Fayyaa Waliigalaa, Daaypartimenti Saayinsii Laaboratooraa Tibbaa.

*Gaaffilee haala ummataa (demographic) fi sababni walqabatu MRSA wajjiin namoota daarua cirurgii keessatti galmaa'an Asella referral and teaching hospital keessatti.

Qoqa 1: Haala Ummataa-Fi-Hawaasaa (Socio-Demographic) Qooddatoon Qorannoo**

1. Umurii _____

2. Saalaa

a. Dhiira

b. Dhalaa

Qoqa 2: Seenaa Nam-tijannaa Dura Galmaa'uu**

- Guyyaa mana fayyaa keessatti galmaa'amuu: dd/mm/yy

1. Sila torban 6 (jira) dura mana fayyaa keessatti galmaa'uu qabduu?

A) Eeyyee

B) Lakki

- Yoo deebii 1 irratti "Eeyyee" ta'e, guyyaa meeqa mana fayyaa keessatti turte? _____

2. Sila torbee shan jiru dura meeshaa fayyaa (fkn Kaateetaraa, laayinii dhangala'aa, fa'i) keessa fayyadamtuu ta'uu qabdaa?

A) Eeyyee

B) Lakki

3. Sila torbee shan jiru dura antibiotikii fudhattee jirtaa?

A) Eeyyee

B) Lakki

4. Sila madaa dheeraa (chronic wound) qabdaa?

A) Eeyyee

B) Lakki

5. Sila torbee jiru dura cirurgii (surgery) ta'uu qabdaa?

A) Eeyyee

B) Lakki

- Yoo deebii 5 irratti "Eeyyee" ta’e, maaliif ta’e? _____

6. Sila torbee shan jiru dura antibiotikii dhangalaafame (prescribed) fudhattee jirtaa?

A) Eeyyee

B) Lakki

- Yoo deebii 6 irratti "Eeyyee" ta’e, antibiotikii dhangalaafame maal ta’e? _____

Qoqa 3: Haala Galmaa’uu Booda**

1. Guyyaa meeqa mana fayyaa keessatti turte? _____

2. Sila guyyaa 48 (dabran) keessatti dhukkubaa haaraa (infection) dhagahamee qabdaa?

A) Eeyyee

B) Lakki

- Yoo deebii 2 irratti "Eeyyee" ta’e, maaliif ta’e? _____

3. Sila seenaa dhiiga dhiyeessii (blood transfusion) qabduu?

A) Eeyyee

B) Lakki

Annex 3: Procedure for specimen collection, processing and result interpretation

Media Preparation, Procedure for Specimen Collection and Processing

- ❖ Collection and processing of Nasal swab

1. With a sterile cotton swab moistened with sterile normal saline gently swab the inside of noses

2. Label the sample with the patient code number and send to lab as soon as possible

3. Inoculate the specimen in to mannitol salt agar and blood agar aseptically

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

5. Examine and report the culture; look for colony characteristics and perform biochemical test

6. Determine drug susceptibility pattern of the isolated organism

- ❖ Preparation of culture media

Blood Agar

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

Preparation of blood agar

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

2. Weigh 40g of Blood Agar Base.

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

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

5. Autoclave at 121°C for 15 minutes.

6. Cool to 45-50°C and aseptically add 50ml of sterile defibrinated blood.

NB: Blood is taken from sheep from jagular vein and collected to a vessel which contains beads that defibrinate the bood

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

8. Cover the petri-dishes and allow the blood agar to coagulate before storage in refrigerator.

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

Mannitol Salt Agar (MSA)

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

Preparation of mannitol salt agar

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

2. Weigh 111g of Mannitol salt agar powder.

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

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

5. Autoclave at 121°C for 15 minutes.

6. Cool to 45-50°C for dispense

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

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

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

Mueller Hinton Agar (MHA)

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

Preparation of Mueller Hinton Agar

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

2. Weigh 21g of mueller hinton agar powder.

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

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

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

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

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

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

- Biochemical testing procedures

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

Procedure for Catalase test

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

Principle: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it in to contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

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

2. Using a sterile wooden stick take the organism and immerse into the hydrogen peroxide solution

3. Look for immediate bubbling

4. Interpretation

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

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

Procedure for Coagulase test

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

Principle: In the presence of the enzyme coagulase, the addition of commercial rabbit plasma

produces a clumping reaction.

1. Place a drop of physiological saline on two separate slides

2. Emulsify the test organism in each of the drop to make thick suspension

3. Add one drop of plasma to one of the suspensions and mix gently

4. Look for clumping of the organism within 10 seconds

5. Interpretation

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

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

Procedure for DNAse test

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

1. Divide a DNA-ase plate into the required number of strips by marking underside of the plate

2. Using a sterile loop or swab, spot-inoculate the test and control organisms

3. Incubate the plate at 35–37oC overnight

4. Cover the surface of the plate with 1 mol/l hydrochloric acid solution

5. Look for clearing around the colonies within 5 minutes of adding the acid

6. Interpretation

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

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

Antimicrobial Sensitivity Testing Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth

2. Match the turbidity of suspension with turbidity standard (0.5 MacFarland standard)

3. With a sterile swab take sample from the suspension (squeeze the swab against the side of test tube to remove the excess fluid).

4. Spread the inoculums evenly over the Muller-Hinton agar plate with the swab

5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate

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

7. Measure the radius of the inhibition zone by caliber

8. Interpret :-**Sensitivity (S):** Zone of radius is wider than, equal to, or not more than 3mm smaller

than the control.

Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.

Resistant (R): No zone of inhibition or zone radius measure 2mm or less.