Prevalence and Antimicrobial Resistance Profile of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Factors Associated with it Among Patients with Wound Infections at Werabe Comprehensive Specialized Hospital, Central Ethiopia Region, Ethiopia

A Research thesis submitted to the Department of Microbiology, Immunology and Parasitology (DMIP), College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements for the degree of Master in Medical Microbiology

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ABBREVIATIONS AND ACRONYMS

AR	Antibiotic resistance
CER	Central Ethiopia Region
CLSI	Clinical and Laboratory Standards Institute
MDR	Multidrug resistant
MHA	Muller Hinton agar
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol salt agar
MSSA	Methicillin susceptible *Staphylococcus aureus*
SPSS	Statistical Package for the Social Sciences
WCSH	Werabe Comprehensive Specialized Hospital
WHO	World Health Organization

ABSTRACT

Background: *Staphylococcus aureus* is a highly significant pathogen for global public health because of its extensive distribution, potential to cause infections that could be fatal, and continuous development of drug resistance. In settings such as the community and health care institutions, it is among the frequent causes of wound infection. Worldwide, the occurrence of drug-resistant *S. aureus*, particularly MRSA, has surged at a concerning rate. Nonetheless, prior research in Ethiopia and other nations found notable regional differences in the incidence and associated variables.

Objective: To determine MRSA's prevalence and pattern of antimicrobial susceptibility as well as to assess factors associated with MRSA among patients with wound infection at Werabe Comprehensive Specialized Hospital, Werabe City, CER, Ethiopia.

Methods: A cross-sectional study centered in a hospital was carried out from June 2023 to February 2024 G.C. *S. aureus* was isolated from a total of 384 wound swabs. Standard laboratory techniques were used to identify and culture *S. aureus*. For the identification of MRSA, testing for antimicrobial susceptibility was carried out in accordance with CLSI guidelines. Socio-demographic information as well as other potential contributing factors was gathered using a structured questionnaire. SPSS version 27 was used for analysis once the data were entered into Epi-info. The study employed descriptive statistics and the Logistic regression analysis, both multivariate and bivariate. To evaluate the association between the variables that are dependent and independent, the odds ratio and 95% confidence interval were calculated, with a P value of less than 0.05 deemed statistically significant.

Results: Of the 384 participants in the study with wound infections, 90 (23.4%, 95% CI: 19.3-28.0) had *S. aureus* isolated from them. Twenty-four (26.7%, 95% CI: 19-37) of the 90 *S. aureus* isolates were found to be MRSA. While the majority of the isolates (87.8%) were resistant to penicillin, Linezolid worked against every strain of *S. aureus*. All 24 of the isolates of MRSA had penicillin resistance. All (100%) of the MRSA isolates and twenty-eight (31.1%) isolates of *S. aureus* were multidrug-resistant (MDR). Various characteristics were evaluated for potential association with MRSA; however, only hospital stays longer than 72 hours (P=0.031, AOR: 3.06, 95% CI: 1.11-8.47) showed statistically significant association with MRSA.

Conclusion: The study showed significant prevalence of *S. aureus* (23.4%) and MRSA (26.7%) in patients with wound infection at WCSH. Both *S. aureus* and MRSA showed the highest resistance to penicillin but 100% sensitivity to linezolid.

Key words: Wound infection, *S. aureus,* MRSA, MDR, Werabe

1. INTRODUCTION

1.1 Background

A wound is a skin incision through which subcutaneous tissue is exposed. The breakdown of skin integrity resulting from a wound creates an environment that is suitable for microbial development, as it is warm, moist, and rich in nutrients (1). One of the main factors of hospital stays is infection from these injuries. Bacterial growth in wounds is linked to wound severity and a longer healing period (2). Globally, the main factors of morbidity and mortality among people are infections of wounds. In particular, surgical site infections account for around 25% of nosocomial infections and are the third most often reported kind of infection(3). *S. aureus* is one of the known bacteria to cause infection and it is linked to a rise in drug-resistant isolates, among other bacteria that cause illness (4).

Staphylococcus aureus (S. aureus) is a gram-positive, spherical coccus with a diameter of about 1μm in Gram-stained smears that typically forms irregular grape-like clusters. Not only is it facultatively anaerobic, not-motile, and not-spore-forming, but it can also make coagulase and catalase. More than 50 species and subspecies of staphylococci can be found in water, sewage, dust, air, environments, humans, and living things(5).

Wounds and *Staphylococcus* species infections are frequently linked, particularly in hospitalized patients. In addition to being a risk factor for Methicillin-resistant *Staphylococcus aureus* (MRSA) infection, wounds may be the source of bacteria that cause cross-contamination. The possibility that *S. aureus* colonizes the nose before spreading to wounds highlights the significance of avoiding cross-contamination in hospital settings, particularly while caring for elderly patients. To aid in the recovery of patients and lower healthcare expenses, wounds should be carefully maintained to stop spread of microbes(2).

The strain of *S. aureus* identified as MRSA is characterized as any strain that acquired or developed numerous drug resistance mechanisms against beta-lactam antibiotics, including some penams (penicillin derivatives like methicillin and oxacillin) and cephems like cephalosporins, either by horizontal gene transfer or natural selection(6). *S. aureus* that lacks sensitivity to methicillin is called MRSA. The methicillin group has long been regarded as the preferred

medication for treating infections caused by β-lactams and other antibiotic-resistant *S. aureus* (7).

MRSA's resistance to β-lactams is attributed to a mobile genetic element termed the staphylococcal cassette chromosome (SCCmec), which contains the mecA or mecC gene. Penicillin-binding proteins produced by both mec genes have a much lower affinity for almost all of the beta-lactam antibiotics(8). Furthermore, penicillin-binding protein 4 (PBP4) has been shown in recent research to be a significant predictor of methicillin resistance in *S. aureus*, especially in community-acquired MRSA strains(9).

1.2 Statement of the problem

Antibiotic resistance (AR) occurs throughout the world affecting thousands of people. WHO states that AR is a big challenge to global health and its level is increasing at an alarming rate. As a result of AR, different diseases are becoming more difficult to treat(10). Because MRSA is multidrug-resistant and can spread epidemics in hospital and community settings, effective treatments are required, including surveillance for prevention and suppression(11).

S. aureus is a prevalent pathogen that can infect humans as well as a variety of animal species. Due to its extensive distribution, the potential to produce lethal infections, and the continuous development of drug resistance, it belongs among the diseases with the highest global significance for public health(12). One of the most common illnesses in the world today is MRSA. These same commensal bacteria that develop in healthcare and community settings are among the primary causes of bacterial infection, endocarditis, infections of the soft tissues and skin, bone and joint infections, and hospital-acquired infections. The main feature of MRSA epidemiology is the recurrent evolution of epidemic strains. Because MRSA consistently results in high rates of morbidity and fatality, it remains a severe clinical danger even in locations where its prevalence has recently decreased. To achieve successful therapy, which is still difficult to get, it is necessary to assess novel antibiotics (13).

Over the past 20 years, there has been a steady rise of MRSA infections in healthcare facilities, which is now a serious global public health concern. Parallel to this, it has been identified as one of the most prevalent and invasive microbes connected to infections in hospitalized patients. It is

currently regarded as one of the most important antimicrobial-resistant bacteria and a danger to public health (14). The emergence of new resistance mechanisms and inappropriate antibiotic usage that is, the use of antibiotics without a prescription, excessive prescriptions from medical professionals, and overuse by the general public exacerbate this issue even more. As a result, the WHO designated MRSA as a high-priority pathogen for research and the development of new antibiotics (15). However, there is a lack of coordination and harmonization in the general epidemiology of multidrug-resistant bacteria, especially in developing nations like Ethiopia(16). Because these bacteria can develop resistance, which can result in a longer hospital stay and more challenging treatment, understanding how these microbes colonize hospital environments is crucial(2).

All WHO regions have reported that MRSA prevalence is higher than 20%, while national reports from nine African nations have indicated that prevalence ranges from 12% to 80%(17). MRSA frequency in Ethiopia varies from 28.3% in Dessie(18) to 82.3% in Arbaminch (4) making it a public health concern. Furthermore, the highest prevalence of MRSA (40.4%) was found in Southern Ethiopia when the regional prevalence of MRSA was also computed, which was nearly two times greater than the Tigray region's result (20.3%)(16).

The CDC estimates that in the United States in 2017, there were 323,700 MRSA cases in 4 hospitalized patients, 10,600 deaths, and 1.7 billion dollars in medical expenses(19). The estimated mean hospitalization expenditures, duration of stay, and death in hospital for the anti-MRSA medication group in Japan were US$33 548, 75.7 days, and 22.9%, respectively; these numbers were 3.43, 2.95, and 3.66 times higher than those for the control group. After accounting for the 1584 institutions, the total incremental cost of MRSA was estimated to be US$2 billion (3.41% of total hospitalization expenses), 4.34 million days (3.02% of total length of stay), and 14.3 thousand fatalities (3.62% of total mortality)(20). AMR-related mortality linked to MRSA surpassed 100,000 in 2019, according to a study carried out in 204 countries(21).

There is a noticeable geographic diversity in the MRSA burden because of several variables, such as distinct regional infection control procedures and pathogen-specific traits of the circulating clones(22). Because MRSA infections are resistant to many drugs, they are often

difficult to treat (23). According to earlier research, *S. aureus* has become infamously resistant to practically every antimicrobial medication used in Ethiopia, including sulphonamides, penicillin, vancomycin, tetracyclines, cephalosporins, and methicillin(24).

Methicillin resistance is an indicator of resistance to several beta-lactam antibiotics, which include the majority of cephalosporin antibiotics and penicillin antibiotics, two of the most widely used antibiotics. MRSA can become resistant to numerous other widely used antibiotics, including erythromycin, clindamycin, gentamycin, ciprofloxacin, and fusidic acid, in addition to beta-lactam antibiotics(25). Hence, methicillin resistance is a sign of resistance to a variety of antibiotics, even if methicillin is not being used widely. MRSA can be obtained in the community or a hospital. It should be noted that community-acquired MRSA has a different antibiotic resistance profile than hospital-acquired MRSA(6).

MRSA is a serious and persistent challenge to the treatment of infection, is a major human pathogen globally, and a global healthcare issue(9), and its prevalence has been rising at an alarming rate across the globe. However, findings from research conducted both internationally and in Ethiopia have demonstrated significant regional variations in prevalence and related factors(16). The variation in antibiotic resistance rates between different regions shows how antibiotic resistance patterns vary by geographic and regional location as well as throughout time (4). Since factors mentioned earlier such as treatment modalities and practices, patient adherence behaviors and others are different across regions and specific, it creates inconclusive finding to our study site. Thus, the purpose of this study is to ascertain the prevalence of MRSA and the factors that are associated with it in patients who have wound infections attending Werabe Comprehensive Specialized Hospital.

1.3 Significance of the study

There is a wide range of infections caused by MRSA, and they are linked to more severe consequences. Health professionals who are in charge of patient treatment and timely detection of the growth of resistant bacteria will benefit greatly from an investigation into the prevalence of these diseases and their sensitivity patterns, as this will provide up-to-date information.

Monitoring the prevalence of MRSA over time is crucial to halting its spread and thinking about new treatment options. Information on MRSA from southern Ethiopia is scarce. The purpose of this study was to ascertain the prevalence of MRSA and the risk variables that are linked to it among WCSH patients who had wound infections. To help various stakeholders, including the Regional Health Office and WCSH, take appropriate action or implement interventions aimed at reducing the incidence of MRSA, it will offer baseline data about the MRSA burden in the research area. Additionally, because their treatments were specific, WCSH patients with wound infections benefited based on the antibiotic susceptibility test results. Furthermore, the study's findings may act as a foundation for subsequent research and offer more information for addressing the issue.

2. LITERATURE REVIEW

2.1 General characteristics

The Staphylococcus genus is made up of Gram-positive, with spherical cocci that have a diameter of approximately 1 μm. Under a microscope, these organisms appear in clusters similar to grapes or as pairs, short chains. Additionally, they are facultatively anaerobic, not mobile, non-spore-forming, and positive for catalase. Over 50 species and subspecies of Staphylococci are found in water, air, dust, waste, environments, human beings, and animals as well(5).

Humans may get a variety of infections from *S. aureus*. Skin and soft tissue are the most often affected areas; infections in these areas can appear as folliculitis, impetigo, mastitis, infection of the wound, staphylococcal scalded skin syndrome, and furuncles and carbuncles. Bacteremia, also pneumonia, endocarditis, infections of the bones and joints, and the condition known as toxic shock syndrome are examples of more severe infections. *S. aureus* may potentially be the cause of food poisoning outbreaks(26).

2.2 Pathogenesis and virulence factors associated with *S. aureus* infections

Antibiotic resistance, enterotoxogenicity, development of biofilm, and other virulence factors such as lipases, hyaluronidase, adhesion factors, nucleases, proteases, and collagenase secretions are what contribute to *S. aureus*'s pathogenicity. Furthermore, *S. aureus* is growing in significance due to its resistance to popular medications, such as methicillin. *S. aureus* infections resistant to β-lactam antibiotics have been treated with methicillin. Because of these traits, the microbe was getting close to becoming one of the world's most resistant pathogenic bacteria. The MRSA spread has become a major global health concern due to its emergence and rising intensity(16)

Given the strong relationship between virulence factors and antibiotic resistance, the mec gene cassette also carries a number of virulence factors. The Staphylococcal cassette chromosome (SCC) may have many genes encoding different virulence factors in a single horizontal gene transfer. This makes the *S. aureus* strains resistant to methicillin more virulent than other *S. aureus* strains(27).

2.3 Diagnosis

Methicillin-resistant *S. aureus*, like all other *S. aureus*, is a spherical (coccus), Gram-positive bacterium with a diameter of roughly one micrometer. It is not motile and does not produce spores. It usually occurs in chains or clusters resembling grapes and it produce catalase, coagulase and Dnase. MRSA is a slow-growing bacterium that can grow on a range of media, unlike methicillin-susceptible *S. aureus* (MSSA), which has been discovered to occur in mixed colonies with MSSA. Generally lacking in MSSA, the mecA gene (which confers resistance to several antibiotics) is always present in MRSA; occasionally, however, the mecA gene is present in MSSA but not expressed. The most accurate technique for distinguishing MRSA strains is polymerase chain reaction (PCR) testing. In certain situations, a specialized culture medium can be utilized to detect particular strains of MRSA that are resistant to distinct antibiotics. These media have been designed to help distinguish MSSA from MRSA more effectively(28).

2.4 Prevalence of MRSA among wound patients

Various research conducted worldwide has produced diverse MRSA prevalence rates. According to an Iranian meta-analysis study, among culture-positive cases, the prevalence of MRSA infections in burn patients was 77.9% (95% confidence interval [70.2-84]((29). According to an Indian meta-analysis, the pooled proportion of patients infected with MRSA was 26.8% (95% CI: 23.2%-30.7%). Male patients had a higher prevalence of MRSA infection (60.4%; 95% CI: 53.9%-66.5%) than female patients (39.6%; 95% CI: 33.5%-46.1%). In contrast, adult patients (18 years and above; 32%; 95% CI: 5%-80%) had a higher prevalence of MRSA infection than pediatric patients (0-18 years; 68%; 95% CI: 20%-94.8%). It was discovered that the degree of heterogeneity was substantial(30). Out of 25 *S. aureus*, a Brazilian investigation found that 8 (32%) had MRSA(2).

Between 2002 and 2007, the prevalence of MRSA rose from 16% to 41% in Tunisia, while it was only 31% in Libya. Between 2007 and 2011, the incidence in South Africa dropped from 36% in 2006 to 24%. Between 2000 and 2007, the incidence in Botswana ranged from 23 to 44%. Between 2003 and 2005, the prevalence was 52% in Egypt and 45% in Algeria. The northern region of Nigeria had a higher frequency than the southern. The percentages were 39% in the Ivory Coast and 55% in Ethiopia. Except South Africa, the majority of African nations had

lower than 50% MRSA prevalence; however, since 2000, the percentage appears to have increased across the continent(31). In Asmara, Eritrea, two referral hospitals provided 130 individuals for a cross-sectional study. 82 (63.1%) of the 130 samples had *S. aureus* isolated from them, and 59 (72%) of the isolates had MRSA(32).

The pooled culture positive was determined to be 70.0% (95% CI: 61, 79%) in an Ethiopian systematic review and meta-analysis based on a total of 21 studies with 4284 wound samples. The pooled prevalence of *S. aureus* among the recovered bacterial isolates was 36% (95% CI: 29, 42%), of which 49% were resistant to methicillin(3). In a cross-sectional investigation involving 378 patients 67 (37.43%) of the 179 *S. aureus* samples from postoperative and burn wounds at Yekatit 12 Hospital Medical College in Addis Abeba between September 2013 and August 2014 were MRSA(33). Twenty-six (28.3%) were MRSA of the 92 *S. aureus* isolates that were obtained during an additional cross-sectional study at Dessie Referral Hospital in Ethiopia, and among patients with wound infections, 9.8% was the total prevalence of MRSA (18). MRSA prevalence was found to be 82.3% in another cross-sectional investigation conducted in April and June 2019 at Arbaminch Hospital in Ethiopia(4). Five (8.8%) of the 57 (23.2%) wound swabs collected in Hawassa contained *S. aureus*, which was later determined to be MRSA(32).

S. aureus was recovered from 73 (39.7%) of the 184 surgical patients who had developed surgical site infections, according to a study from Debre Markos Referral Hospital in Ethiopia. 36 (49.7%) of the 73 *S. aureus* isolates were MRSA. 19.6% of research participants had MRSA, according to the findings(34). Nonetheless, a 2021 research conducted in the same hospital reported that 32/71 *S. aureus* (45.1%) had MRSA prevalence(35). According to a study conducted at four referral hospitals in Ethiopia, 24.5% of the *S. aureus* isolates had MRSA(36).

2.5 Associated factors of MRSA

Studies reporting related factors for MRSA among individuals with wound infections are insufficient. A limited number of research that identified the contributing elements reported varying factors linked to MRSA.

A study conducted at four referral hospitals in Ethiopia found patients aged 61 and older had a 3.7-fold increased risk of MRSA SSI incidence (AOR = 3.729 (1.179–11.791) when compared to

patients aged 60 and younger. And, individuals who stayed in the hospital for more than 7 days had a relative risk of 1.9 times higher likelihood of contracting MRSA (AOR = 1.856 (0.688–5.311)). Additionally, the probability of getting MRSA was 3.7 times greater in patients with a history of antibiotic usage (AOR = 3.692 (1.059–2.800)) compared to MSSA(36). A Dessie Referral Hospital study found that compared to their counterparts, patients in the inpatient department and farmers had 3.6 (AOR 3.56, 95% CI (1.429–8.857)) and 13.9 (AOR 13.89, 95% CI (4.919–39.192)) times higher risk of developing MRSA wound infection, respectively(18). Similarly, a study at DMRH discovered a statistically significant association between MRSA and longer than 72 hours stays in hospital (AOR 7.402, 95% CI 1.50–239.49), the depth of wound (AOR 4.38, 95% CI 1.049–18.29), the prior wound infection history (AOR 24.33, 95% CI 3.77–156.89), and current use of antibiotic(AOR 7.9, 95% CI 1.44–43.39). However, there was no significant association between MRSA and socio-demographic variables(35). Similarly, MRSA did not significantly correlate with age and sex in Addis Ababa(33) but a different study found that age—specifically, falling within the 19–29 and 30-39 age groups—did associate strongly with MRSA(14).

Conversely, a study in Arbaminch Hospital revealed that any of the factors assessed had no a statistically significant association with MRSA(4) and A study conducted in Hawassa City revealed that various factors were evaluated for potential associations with MRSA, however, all of them failed to found statistically significant associations(37). In a different study conducted at Hawassa University Comprehensive Specialized Hospital, an important proportion of patients who had previously been admitted to the surgical ward had MRSA colonization(38).

2.6 Types of Wounds

The two main categories of wounds are acute and chronic. Acute wounds are those that result from external damage to skin that is still intact; these include surgical wounds, bites, burns, small cuts, and abrasions. Severe traumatic wounds include lacerations and wounds from crush or gunshot injuries (46).

In marked contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue. Pathophysiological abnormalities that may predispose to the formation of

chronic wounds such as leg ulcers, foot ulcers, and pressure sores include compromised tissue perfusion as a consequence of impaired arterial supply (peripheral vascular disease) or impaired venous drainage (venous hypertension) and metabolic diseases such as diabetes mellitus. Advancing age, obesity, smoking, poor nutrition, and immunosuppression associated with disease (e.g., AIDS) or drugs (e.g., chemotherapy or radiation therapy) may also exacerbate chronic ulceration (45).

2.7 Prevention and treatment of MRSA

The most common gram-positive cocci found in wound infections have been staphylococci, with *S. aureus* being the most common isolate. The high prevalence of MRSA isolates in hospital settings is also alarming(3). Physicians are facing a growing challenge in treating methicillin-resistant Staphylococcus bacteremia since it can result in potentially fatal situations. While vancomycin and daptomycin are advised by IDSA as first-line treatments for MRSA, there are a number of disadvantages that call for the development of alternatives that will improve clinical success rates while posing fewer risks. Combining vancomycin/daptomycin with β-lactams has been shown in numerous studies to accelerate bacterial clearance and reduce the risk of 30-day death, making it a potentially effective option(39). *S. aureus* in particular is a pathogen of concern with multiple resistance mechanisms that make treatment difficult. Amoxicillin/clavulanate, ampicillin/sulbactam, and cloxacillin are generally the most often prescribed medications for treating *S. aureus* infections, while levofloxacin, vancomycin, linezolid, and tigecycline are more effective in treating MRSA strains(3).

Gaps Identified

There is a big difference between studies regarding the prevalence of MRSA across different geographical areas even in the same region and inconsistency in different factors to be associated with MRSA. Therefore, it is difficult to generalize about the prevalence of MRSA and the associated factors. So, I found it important to study the situation in our study area.

Conceptual framework

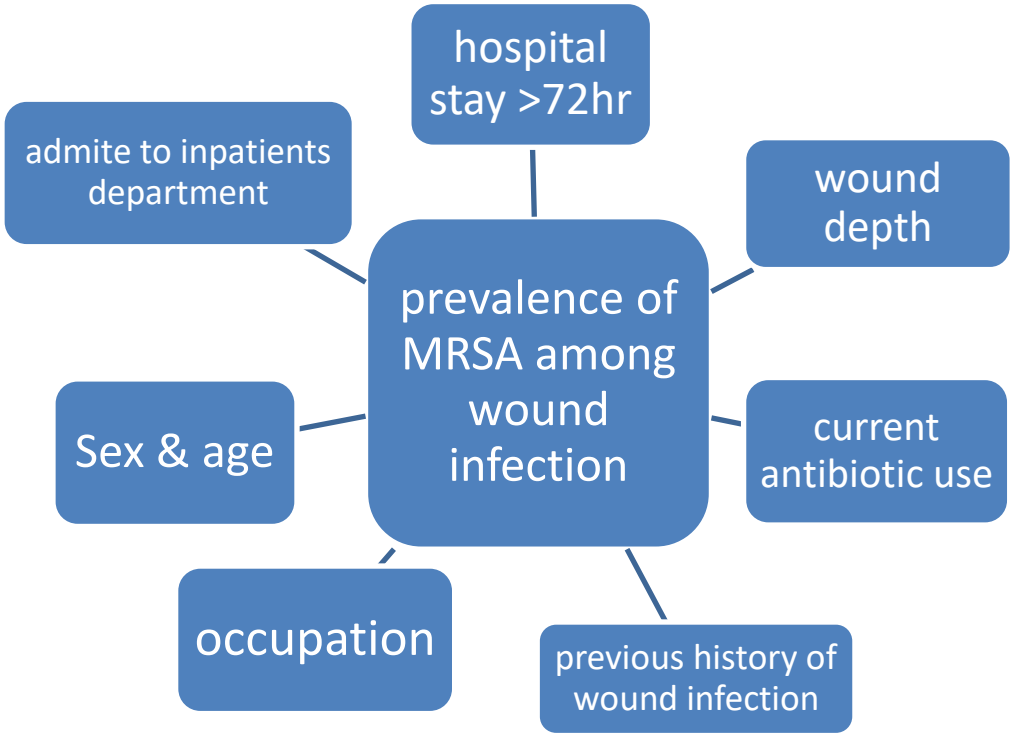

Figure 1: Conceptual frame on possible risk factors associated with prevalence of MRSA among wound infection

3. OBJECTIVE

3.1 General objective

- To determine the prevalence of methicillin-resistant *Staphylococcus aureus* and associated factors among patients with wound infections at Werabe Comprehensive Specialized Hospital, Werabe City, CER, Ethiopia in 2023.

3.2 Specific objective

- To determine the prevalence of MRSA among patients with wound infection at Werabe Comprehensive Specialized Hospital, Werabe City, CER, Ethiopia.
- To determine the antimicrobial susceptibility pattern of MRSA isolates
- To assess factors associated with MRSA among patients with wound infection at Werabe Comprehensive Specialized Hospital, Werabe City, CER, Ethiopia.

4. MATERIALS AND METHODS

4.1 Study Area

Werabe Comprehensive Specialized Hospital, Werabe town, Siltie zone, CER, was the study's site. The settlement of Werabe is situated 60 km north of Hosanna, 202 km north of Hawassa, and 172 km south of Addis Ababa (fig 2). On November 29, 2014, Werabe Comprehensive Specialty Hospital was founded. There are currently over 890 workers, of which 31 are senior specialists, around 72 general practitioners, over 235 nurses, 36 work in medical laboratory technology, three of them are certified medical microbiologists, and over 499 clinical workers in total. Medical specialties include internal medicine, pediatrics, child health, neonatal intensive care unit, surgical procedure, gynecology & obstetrics, orthopedic surgeries, neurological surgery, urology, mental health care, ophthalmology, dermatology, and STDs. Additionally, radiology services.(CT scan, x-ray, MRI, ultrasound, echocardiography, etc.), oncology, intensive care unit, pharmacy services, and laboratory services are among the general, specialty, and subspecialty levels of care provided by WCSH.

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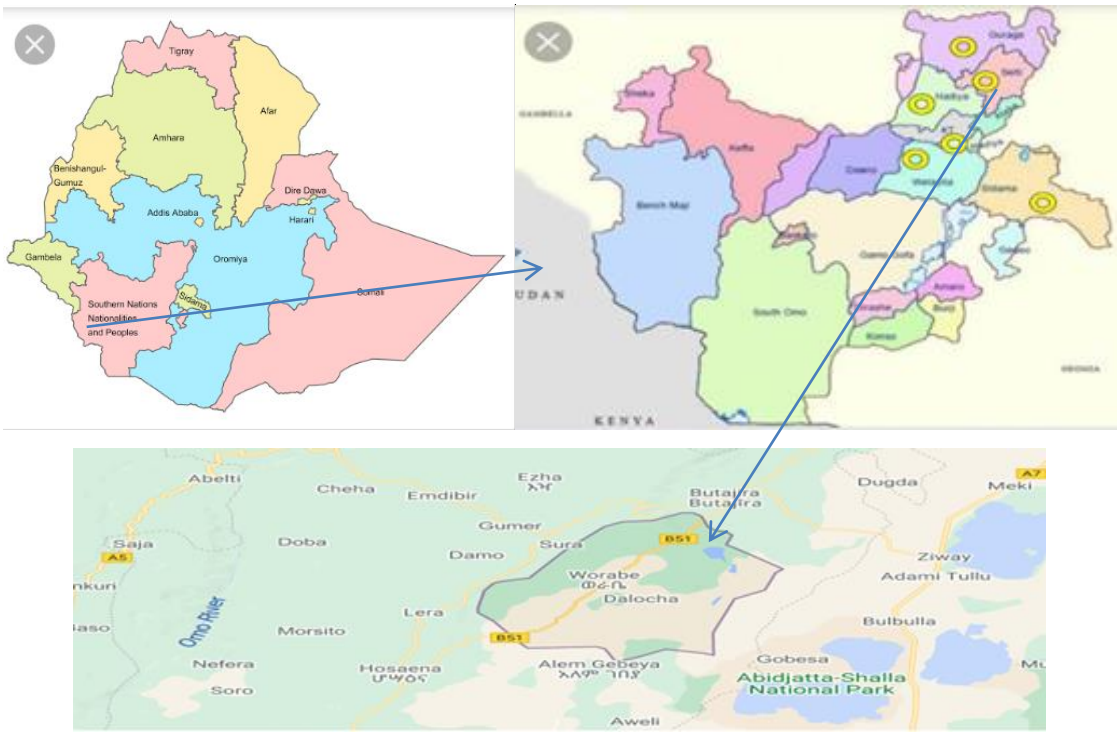


Fig. 2: Map of the study area (Werabe city), Source: Google, January 2023

4.2 Study design

Institution-based Cross-sectional study design was employed from June 2023 to Feb 2024.

4.3 Population

4.3.1 Source population

All patients with wound infection visiting/admitted to emergency departments, OPD, orthopedic (inpatient and outpatient), gynecology, plastic surgery, and general surgical unit of WCSH

4.3.2 Study population

All age groups with wound infections visited or admitted to the orthopedic (inpatient and outpatient), adult and pediatric emergency departments, outpatient department, surgery for plastic surgery, gynecology, and general surgery unit of WCSH during the study period

4.4 Eligibility criteria

4.4.1 Inclusion criteria

- All age-group patients who were willing to engage in the research study and had an open or closed wound infection were included.

4.4.2 Exclusion criteria

- Patients who were on antibiotics for the last two weeks
- Patients unable to provide consent or assent such as unconscious patients were not part of the study,
- Individuals who had wound infections and whose wounds had been dressed, cleaned, and disinfected before sample collection

4.5 Variables

4.5.1 Dependent variable

Prevalence of MRSA, Antimicrobial susceptibility profile of MRSA

4.5.2 Independent variable

Socio-demographic factors, past antibiotic use history, prior hospitalization history, wound infection history, type of wound, depth of wound; length of stays in the hospital, and co-morbidities.

4.6 Sampling

4.6.1 Calculation of Sample Size

The sample size was determined by the formula for a single population proportion using a prevalence of 49% from a previous study (3) and assuming a 95% confidence interval ($Z\alpha/2 = 1.96$).

$$N = \frac{(Za/2)^2 p(1-p)}{d^2}$$

P = prevalence from prior study, P=0.49, n= sample size, d = margin of error,

$= Z_{1-\alpha/2}$ (Z value at 95% confidence interval) = 1.96, d = 0.05.

$$\frac{(1.96)^2 * 0.49 * 0.51}{(0.05)^2} = 384$$

Accordingly, 384 patients participated in the study.

4.6.2 Sampling technique

The study employed a convenient sampling technique to choose participants, and samples were obtained from consecutive individuals with wounds until the required size of the sample was reached over the period of the investigation.

4.7 Data collection tools and procedure

From various literature sources structured questionnaires were derived and utilized to gather data. Once the interview was over, a sample of the wounds from the patients was also obtained. Five WCSH-employed, B.Sc.-holding, skilled, and experienced nurses performed the interview. After giving information about the process in a separate sample collection room, a qualified laboratory technologist collected wound samples using the SOPs that the primary investigator had provided for data collectors. Both the lead investigator and the site supervisor oversaw the

process. The data collectors received training on how to gather information, correctly complete questionnaires, and obtain wound samples using a pretest. Following the interview and wound sample collection, the sample was moved and kept in storage until the analysis was completed in accordance with the SOP prepared for the purpose of this study. The detailed SOP and steps are presented below in the sample collection, transport, and storage section.

4.8 Specimen collection, transportation, and storage

Samples were collected using an aseptic method. Before the sample was collected, the wound was cleansed with sterile normal saline. Using a sterile swab, samples were taken from study participants' wounds by rotating the swab tip over a 1 cm2 region of viable wound for five seconds (Levine method). The wound was sufficiently compressed to release fluid, which was then transferred to a sterile test tube and labeled. After the skin was cleaned with 70% alcohol, samples from closed wounds were obtained either directly from a drainage tube or via aspiration in the case of an abscess that was cut open and drained to receive medical treatment. At one point in time, multiple wound swabs were obtained from each subject to maximize the likelihood of recovering the bacteria. The hospital's microbiology lab received the swab samples immediately, and it processed them as soon as they got there. The samples were kept at between 2 and 8 degrees Celsius if a delay was unavoidable.

4.9 Laboratory investigations

4.9.1 Sample Processing and culturing

Every wound swab sample was inoculated onto 5% sheep blood agar and mannitol salt agar. For up to 24 hours, Every inoculated agar plate was incubated in an aerobic environment at 370C.. The plates were checked for bacterial growth after being incubated for the entire night. Gram staining, a biochemical test, colony features, hemolysis pattern, and growth were all carried out for identification..

4.9.2 Identification of *S. aureus*

Based on colony features on blood agar and MSA, gram reaction, and biochemical test results such as catalase and coagulase tests, bacterial isolates were identified as *S. aureus*. Moreover, identifying methods included mannitol fermentation on MSA and β-hemolysis on blood agar. To distinguish between Staphylococci, which produce the catalase enzyme, and Streptococci, which

do not, a catalase test was performed. The test organism was placed on a slide, and then hydrogen peroxide was added. If the organism produces catalase, hydrogen peroxide is broken down by catalase into oxygen and water, which generates oxygen bubbles.

Coagulase testing was performed on all catalase-positive and gram-positive cocci to distinguish *S. aureus*, which generates coagulase, from other Staphylococci. Because coagulase changes fibrinogen into fibrin, it causes plasma to clot. The majority of *S. aureus* strains produce two different forms of coagulase: Free coagulase, which activates a coagulase-reacting factor found in plasma, converting fibrinogen to fibrin. When doing the tube test, free coagulase is found by clotting. Without the aid of a coagulase reacting factor, bound coagulase (clumping factor) directly transforms fibrinogen into fibrin. The tests used to determine free and bound coagulase were tube and slide coagulase tests, respectively. By emulsifying an organism in physiological saline with plasma, the slide coagulase test was carried out; clumping seen within 10 seconds indicated *S. aureus*. To perform the tube coagulase test, isolated *S. aureus* colonies were emulsified with plasma, incubated for four hours at 37°C, and then the colonies were checked for the formation of clots—any size clot is indicative of *S. aureus*

4.9.3 Antibacterial susceptibility testing

Using the disc diffusion method, antibacterial susceptibility testing was performed on each *S. aureus* isolate as per the Clinical and Laboratory Standards Institute (CLSI, 2022) guidelines. Using nutritional broth, a standardized suspension of every *S. aureus* isolate was made and compared to the McFarland 0.5 turbidity standard. After dipping a sterile swab into the suspension, an excess liquid was wiped out by spinning the swab against the tube's surface. Subsequently, the swab was injected across the whole Muller Hinton agar (MHA) surface. For three to five minutes, the inoculated plates were allowed to dry at room temperature.

On the inoculated MHA plates, a series of antibiotic discs with 30µg of cefoxitin, 5µg of ciprofloxacin, 2µg of clindamycin, 15µg of erythromycin, trimethoprim-sulfamethoxazole (1.25/23.75µg), 15µg of azithromycin, 30µg of linezolid, 10 units of penicillin, 30µg of tetracycline, and 10µg of gentamicin were placed on the inoculated MHA plates. Cefoxitin (30µg) disc, the surrogate drug used for the detection of MRSA, had its incubation period expanded to 24 hours.

4.10 Data quality assurance and Quality control

Five B.Sc. nurses who work at WCSH were instructed data collecting procedures, inclusion and exclusion criteria, swab sample collection, patient information security, and how to interact with and respect the participants' desires. The questionnaire underwent translations into Amharic and back into English, with a consistency check performed. Prior to the actual data collection, the questionnaire was pretested at WCSH on 5% of the sample population. Discussions about issues or challenges related to data collecting were undertaken between the principal researcher and the data collectors when needed.

Data were cleaned, double-entered, and frequency-run after being entered into SPSS (Statistical Package for the Social Sciences) software. The microbiology laboratory strictly adhered as per its standard operating procedures; quality assurance is conducted at the pre-analytical, analytical, and post-analytical stages. Additionally, Instructions from the manufacturer were adhered to. Technical performance was examined as well as the correct use of laboratory reagents. A highly qualified and skilled microbiologist oversaw the analysis in the lab and verified the findings. Strains used for quality control, like *S. aureus* (ATCC-25923), to verify the quality of the antimicrobial discs and culture media. To verify sterility, 5% of the batch of produced culture media was cultured overnight. At the end of each day, the principal investigator verified that all data, including laboratory results, were complete and that any feedback was shared among the data collectors.

4.11 Data processing and analysis

Epi-data version 4.6 was used to code and enter the data. After that, the data were exported to SPSS version 26 for additional cleaning and analysis. Calculations were made for descriptive statistics including frequency, mean, and percentage. Logistic regression and chi-square were used to assess the associations between the dependent and independent variables.

To evaluate associations, a binary logistic regression model was employed. An estimate of the odds ratio (OR) and its associated 95% confidence interval (CI) was made. Candidates for additional multivariable analysis were variables whose bivariable p-value was less than 0.25. Statistical significance was determined for variables with a p-value of less than 0.05, and the

strength of the associations was assessed using an adjusted odds ratio (AOR) with a 95% confidence interval. Data were presented using text, tables, and charts.

4.12 Ethical Consideration

After obtaining ethical clearance from the Departmental Research Ethics Review Committee of the DMIP the study was conducted. The research was approved by WCSH through a formal letter of permission, and from the hospital administration, a support letter was obtained. Each study participant was informed of the purpose and confidentiality of the study, and they were asked to provide written informed consent or assent. Participants' information was kept strictly confidential, and codes were used in place of names to protect any information that could identify them. The data was stored in a secure location and was accessible only by the principal investigator to address questions of the study. The results of the culture and antibacterial susceptibility tests were right away reported to the treating doctor for patient care.

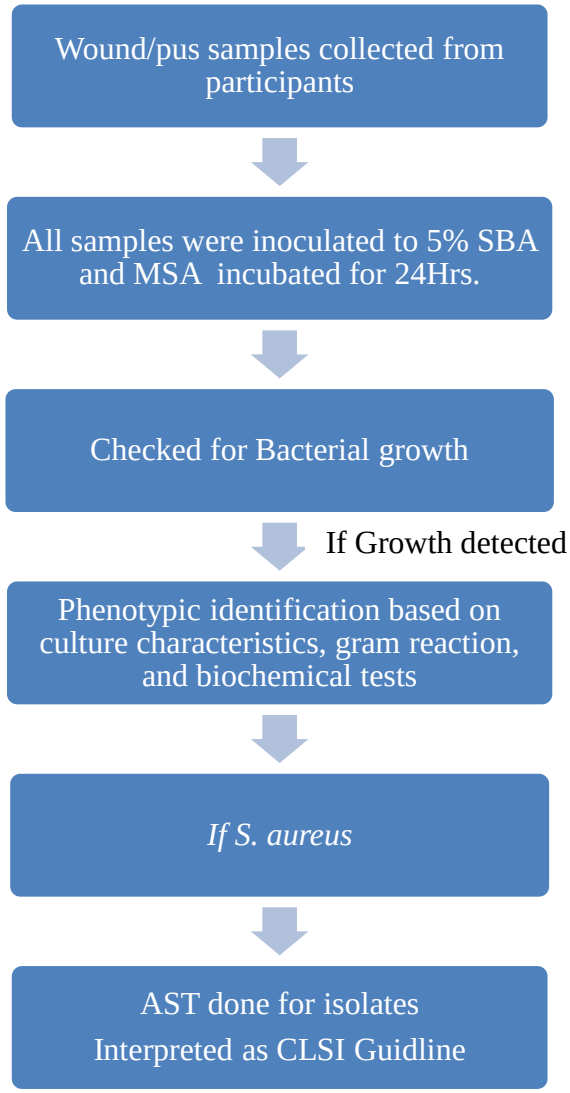
4.13 Dissemination of research findings

The study's thesis will be printed out and stored at the AAU College of Health Sciences Library. The thesis will be presented in an open defense and submitted to the Department of Microbiology, Immunology, and Parasitology. Additionally, the hospital will be informed of the study's findings. The manuscript is ready and will be sent for publication in an international peer-reviewed journal.

4.14 Operational definition

Methicillin-resistant *S. aureus*	*S. aureus* resistant to cefoxitin on MHA after 24 hours of incubation with a zone of inhibition ≤21 mm(40).
Multidrug-resistant *S. aureus:*	*S. aureus* resistant to ≥ three antibiotics belonging to different classes(40).
Superficial infection:	involving the skin or subcutaneous tissue of the incision (41).
Deep infection:	involving the deep soft tissue of the incision (fascial & muscle layers)(41).

Figure 3: Workflow for the isolation of Methicillin-resistant *S. aureus*



5. RESULTS

5.1 Socio-demographic characteristics

This study included a total of 384 patients with wound infections; 268 (69.8%) of them were from rural settings, and 240 (62.5%) were men. The age range of the study participants is from 1 to 80 years. Twenty-nine years old was the median age (interquartile range [IQR]: 16–30). The age group under thirty years old comprised more than half of the participants (216, 56.3%). Table 1 shows that 218 participants, or 56.8%, were married, while 204 participants, or 53.1%, had no formal education (Table 1).

Table 1: Socio-demographic characteristics of patients with wound infection at WCSH, 2023 (n=384)

Variables	Responses	Frequency n (%)
Age (in years)	≤15	66(17.2)
	16-30	150(39.1)
	31-45	116(30.2)
	>45	52(13.5)
Sex	Male	240(62.5)
	Female	144(37.5)
Educational status	No formal education	204(53.1)
	Primary school	114(29.7)
	Secondary school and above	66(17.2)
Occupation status	Farmer	74(19.3%)
	Merchant	52(13.5%)
	Housewife	82(21.4%)
	Others	176(45.8%)
Place of residence	Urban	116(30.2)
	Rural	268(69.8)
Marital status	Married	218(56.8)
	Single	140(36.4)
	Others	26(6.8)

5.2 Clinical Characteristics

Among the 384 study participants who had a wound infection, 52 (13.5%), 73 (19.01%), and 10 (2.6%), respectively, had a prior history of hospital admission, co-morbidities, and prior infections of wound. Of the study participants with co-morbidities, 41 individuals (10.7%) had diabetes.

The majority of research participants (210, 54.7%) spent fewer than 72 hours in the hospital during their most current visit. Based on the depth of the wounds, deep wounds were the most commonly diagnosed (250, 65.1%), while trauma wounds accounted for the majority of the infected wounds (183, 47.7%). A large number of patients (284, or 74%) were from the inpatient ward. Of the participants, 104 (36.5%) were admitted to the orthopedic ward, and among them, 86 (22.4%) and 90 (23.4%) respectively visited the plastic surgery and outpatient department (OPD) wards. A total of 154 (40.1%) participants revealed having previously used antibiotics. Of those, 116 (75.3%) stated that the antibiotics were prescribed by a healthcare professional, and 104 (68.4%) completed the entire dosage (Table 2).

Table 2: Clinical characteristics of wound-infected patients at WCSH, 2023

Variables	Frequency	Percentage
Hospitalization history of previous twelve months (n=384)		
Yes	52	13.5
No	332	86.5
Co-morbidity (n=384)		
Yes	73	19.01
No	311	80.99
Previous wound infection history (n=384)		
Yes	10	2.6
No	374	97.4
Current duration of stays in the hospital (n=384)		
< 72 hours	210	54.7
>= 72hours	174	45.3
Antibiotics use of prior 12 months(n=384)		
Yes	154	40.1
No	230	59.9

Health professionals prescribed antibiotics (n=154)		
Yes	116	75.3
No	38	24.7
Finish the entire dosage (n=154)		
Yes	104	68.4
No	50	31.6
Wound type (n=384)		
Surgical wound	80	20.8
Ulcer wound	103	26.8
Burn wound	16	4.2
Trauma wound	183	47.7
Others	2	0.5
Wound depth (n=384)		
Deep wound	250	65.1
Superficial wound	134	34.9
The hospital's patient setting(n=384)		
Inpatient	284	74.0
Outpatient	100	26.0
Patient admitted Ward (n=384)		
Emergency	16	4.2
Gynecology	2	0.5
Orthopedic ward	140	36.5
OPD	86	22.4
Plastic surgery	90	23.4
Surgical ward	50	13.0

Others includes– animal bites, crush, gunshot injuries

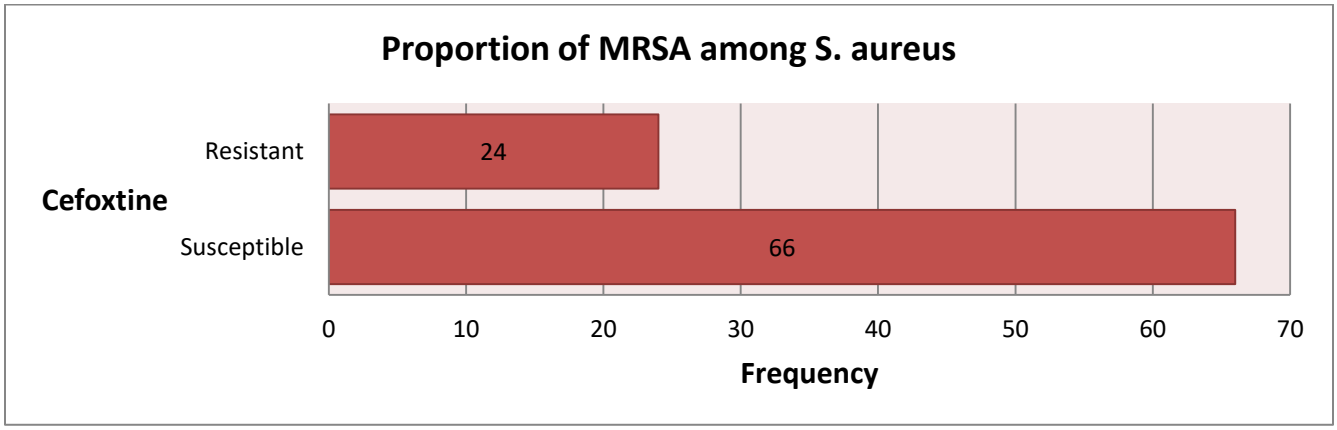
5.3 *S. aureus* prevalence and distribution in relation to clinical and socio-demographic characteristics

S. aureus was isolated from 90 (23.4%, 95% CI:19.3-28.0) of the 384 wound swabs obtained from the study participants. The majority of the *S. aureus* isolates were obtained from patients who were male (62, 68.9%), age group of 15-29 (41, 45.6%), rural residents (64, 71.1%), and those with no formal education (41, 45.6%). Moreover, *the S. aureus* isolation rate was higher

among patients who stayed for 72 hours and longer (50, 55.6%), patients admitted to the Orthopedic ward (36, 40%), and trauma patients (46, 51.1%) (Table 3).

5.4 Proportion of MRSA among *S. aureus* and its distribution in terms of clinical and socio-demographic characteristics

Among the 90 *S. aureus* isolates, 24 (26.7%, 95% CI:18-37) were identified as MRSA (Bar-Chart 1).



Bar-Chart 1. Proportion of MRSA among *S. aureus*

A comparatively large proportion of MRSA isolates were from male patients (20, 83.3%), older than 45 years (8, 33.3%), and rural dwellers (22, 91.7%). Twenty-two (91.7%) of the study participants with MRSA had no history of hospitalization and wound infection in the last 12 months. Majority of the MRSA isolates were from patients who had no history of antibiotic use in the last 12 months (18, 75%) and patients with no co-morbidity. Those who stayed in the hospital for more than 72 hours (18, 75%) were responsible for more than half of the MRSA cases. Half (12, 50%) of the MRSA were isolated from patients with trauma wounds and 6(25%) from patients with non-healing ulcers. Twenty (83.3%) of the patients with MRSA infection were from the inpatient ward, and 16(66.7%) of them had deep wound infections. Half of MRSA (12, 50%) were from wound swabs (Table 4).

5.5 Antimicrobial resistance profile of *S. aureus*

Every strain of *S. aureus* was susceptible to linezolid. The greatest percentage of *S. aureus* resistance to penicillin (87.8%) was found, followed by ciprofloxacin (28.9%), tetracycline (26.7%), and azithromycin (22.2%). Lowest resistance was detected for gentamicin (8.9%),

clindamycin (8.9%) and erythromycin (13.3%), Trimethoprim/Sulfamethoxazole (13.3%)(Table 4)

Table 3: *S. aureus* and MRSA distribution among different variables at WCSH, 2023

Variables	Bacteria isolates		
	***S. aureus* (n=90)**	**MSSA (n= 66)**	**MRSA (n=24)**
Age			
≤14	17(18.89%)	12(18.18%)	5(20.8%)
15-29	41(45.6%)	34(51.52%)	7(29.2%)
30-49	16(17.8%)	10(15.15%)	6(25%)
50-59	6(6.7%)	2(3.03%)	4(16.7%)
>=60	10(11.11%)	8(12.12)	2(8.3%)
Sex			
Male	62(68.9%)	42(63.6)	20(83.3%)
Female	28(31.1%)	24(36.4%)	4(16.7%)
Place of residence			
Urban	26(28.9%)	24(36.4%)	2(8.3%)
Rural	64(71.1%)	42(63.6%)	22(91.7%)
Educational status			
No formal education	41(45.6%)	28(42.4%)	13(54.2%)
Formal education	49(54.4%)	38(57.6%)	11(45.8%)
Occupation			
Farmer	21(23.33%)	8(12.12%)	13(54.2%)
Merchant	8(8.9%)	4(6.06%)	4(16.7%)
Housewife	12(13.33%)	10(15.15%)	2(8.3%)
Others	49(54.44%)	44(66.67%)	5(20.8%)
History of hospitalizationin the last 12 months			
Yes	12(13.3%)	10(15.2%)	2(8.3%)
No	78(86.7%)	56(84.8%)	22(91.7%)
Prior wound infection history in the last 12months			
Yes	4(4.4%)	2(3.03%)	2(8.3%)
No	86(94.6%)	64(96.97%)	22(91.7%)
Previous antibiotic use history inthe last twelve months			
Yes	32(38.6%)	26(39.4%)	6(25%)
No	58(61.4%)	40(60.6%)	18(75%)
Co-morbidity			

Yes	13(14.4%)	12(18.2%)	1(4.2%)
No	77(85.6%)	54(81.8%)	23(95.8%)
Ward patient is admitted			
Emergency	4(4.4%)	4(6.1%)	0(0%)
Orthopedic ward	36(40%)	20(30.3%)	16(66.7%)
Plastic surgery	24(26.7%)	22(33.3%)	2(8.3%)
Surgical ward	10(1.1%)	8(12.1%)	2(8.3%)
OPD	16(17.8%)	12(18.2%)	4(16.7%)
Length of stay at thehospital			
< 72 hours	40(44.4%)	34(51.5%)	6(25%)
≥ 72 hours	50(55.6%)	32(48.5%)	18(75%)
Type of wound			
Surgical	14(15.6%)	10(15.2%)	4(16.7%)
Trauma	46(51.1%)	34(51.5%)	12(50%)
Non-healing ulcer	24(26.7%)	18(22.3%)	6(25%)
Burn	6(6.7%)	4(6.1%)	2(8.3%)
Patient setting			
Inpatient	70(77.8%)	50(75.6%)	20(83.3%)
Outpatient	20(22.2%)	16(24.4%)	4(16.7%)
Depth of wound			
Deep	62(68.9%)	46(69.7%)	16(66.7%)
Superficial	28(31.1%)	20(30.3%)	8(33.3%)
Types of specimens			
Pus	32(35.6%)	22(33.3%)	10(41.7%)
Wound swab	52(57.8%)	40(60.6%)	12(50%)
Abscess	6(6.7%)	4(6.1%)	2(8.3%)

Others includes- students, government employees, daily laborers

5.6 Antimicrobial resistance profile of MRSA

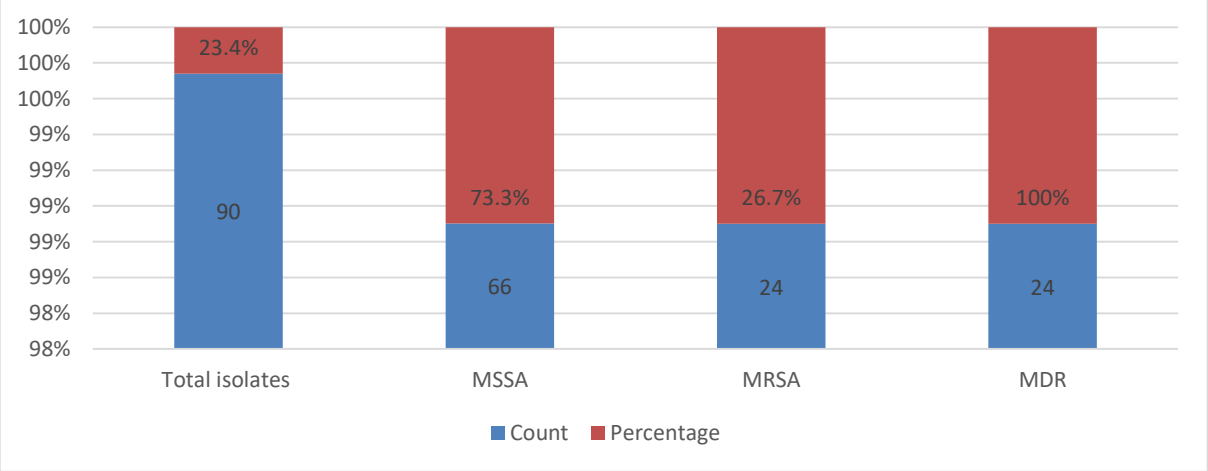
Every single MRSA isolate (24, 100%) showed resistance to penicillin but was susceptible to linezolid. The MRSA isolates showed high resistance to tetracycline (84.6%), Ciprofloxacin (84.6), and Azithromycin (75%). However, the lowest resistance was reported for gentamicin and clindamycin 8(33.3%) (Table 4).

Table 4: *S. aureus* and MRSA antimicrobial susceptibility profile from wound infection at WCSH, 2023

Antibiotics	*S. aureus* (n=90)		MRSA (n=24)	
	S	R	S	R
Cefoxitin,30µg	66(73.3%)	24(26.7%)	0	24(100%)
Ciprofloxacin, 5µg	64(71.1%)	26(28.9%)	4(15.4%)	20(84.6%)
Clindamycin,2µg	82(91.1%)	8(8.9%)	16(66.7%)	8(33.3%)
Trimethoprim/Sulfamethoxazole, 1.25/23.75µg	78(86.7%)	12(13.3%)	14(58.3%)	10(41.7%)
Azithromycin,15µg	70(77.8%)	20(22.2%)	6(25%)	18(75%)
Gentamicin,10µg	82(91.1%)	8(8.9%)	16(66.7%)	8(33.3%)
Linezolid,30µg	90(100%)	0	24(100%)	0
Penicillin,10IU	11(12.2%)	79(87.8%)	0	24(100%)
Tetracycline,30µg	66(73.3%)	24(26.7%)	4(15.4%)	20(84.6%)
Erythromycin,15µg	78(86.7%)	12(13.3%)	12(50%)	12(50%)

5.7 *S. aureus* and MRSA multidrug resistance pattern

Eleven (12.2%) of the 90 *S. aureus* isolates were sensitive to every antibiotic tested. Overall, 28 (31.1%) of the 90 *S. aureus* isolates and the MRSA isolates all became (100%) MDR, meaning they were capable of resisting three or more antibiotic classes(Table 5).



Bar-Chart 2: MRSA, MSSA, and MDR-MRSA distribution in patients with wound infection

Table 5 Antibiogram profile of the MRSA isolates from wound infection at WCSH, 2023

#	Frequency	List of resisted Antimicrobial agents = number
R0	0	
R1	0	
R2	0	
R3	2	CFX+PEN+TTC
R4	0	
R5	5	CFX+PEN+TTC+AZK+GEN=2 CFX+CPR+ SXT+TTC+PEN=1 CFX+CPR+TTC+AZK+ERY=2
R6	9	CFX+ SXT+PEN+TTC+AZK+CPR=4 CFX+CPR+CLN+AZK+ERY+TTC=3 CFX+CPR+PEN+AZK+ERY+CLN=2
R7	4	CFX+CPR+PEN+AZK+ERY+TTC+GEN=2 CFX+CPR+PEN+AZK+ SXT+GEN+TTC=2
R8	3	CFX+CPR+PEN+AZK+ERY+TTC+ SXT+CLN=1 CFX+CPR+ SXT+GEN+PEN+AZK+ERY+TTC=2
R9	1	CFX+CPR+ SXT+GEN+PEN+AZK+ERY+TTC+CLN

Note. R0, R1, R2, R3, R4, R5, R6, R7, R8, R9- sensitive to all, resistance to one, two, three, four, five, six, seven, eight, and nine antibiotics tested, respectively. ***Key:*** CFX: Cefoxitin, PEN: Penicillin, TTC: Tetracycline, GEN: Gentamycin, AZK: Azithromycin, CPR: Ciprofloxacillin, SXT: Trimethoprim/Sulfamethoxazole, CLN: Clindamycin, ERY: Erythromycin

5.8 Factors Associated with MRSA

The sociodemographic traits of research participants were evaluated for potential associations with MRSA colonization. In bivariate analysis, the following factors were associated with MRSA: sex, occupation, and place of residence. All factors with a P-value of less than 0.25 were combined for multivariate logistic regression analysis to reduce the confounding impact; as the results indicated none of the variables had a statistically significant relationship with MRSA (Table 6).

Additionally, a variety of clinical variables were evaluated to determine any associations with MRSA infection. Accordingly, the bivariate logistic regression analysis revealed statistically significant associations for the following variables: length of hospital stay (P=0.005), use of antibiotics in the previous 12 months (P=0.121), wound infection in the last 12 months (P=0.091), and co-morbidity (P=0.089) (Table 4). Multivariate logistic regression analysis was applied to all variables with a P-value of less than 0.25 to prevent the confounding impact. Hospital stays longer than 72 hours were significantly associated with MRSA (P=0.031, AOR: 3.06, 95%CI: 1.11-8.47), according to the results. Compared to patients who stayed less than 72 hours in the hospital, patients who stayed more than 72 hours had a three times higher risk of developing an MRSA wound infection.

Table 6: Bivariate and multivariate analysis of factors associated with MRSA among patients with wound infection at WCSH 2023

Variable		MRSA		COR (95% CI)	P-value	AOR (95% CI)	*P*-value
		Yes (n= 24)	No(n=360)				
Sex	**Male**	20(83.3%)	220(61.1%)	3.18(1.065-9.504)	0.038	2.7(0.5-15.9)	0.28
	Female	4(16.7%)	140(38.9%)	1		1	
Place of	**Urban**	2(8.3%)	114(31.7%)	1		1	
Residence	**Rural**	22(81.7%)	246(68.3%)	5.098(1.18-22.05)	0.029	2.79 (0.518-14.996)	0.232
Co-morbidity	**Yes**	1(4.2%)	72(20%)	5.8(0.77-43.29)	0.089	7.196(0.837-61.86)	0.072
	No	23(95.8%)	288(80%)	1		1	
Wound infection	**Yes**	2(8.3%)	8(2.2%)	4.00(0.801-19.976)	0.091	2.57(0.365-18.094)	0.343
	No	22(91.7%)	352(87.8%)	**1**	**1**		
Use of antibiotics	**Yes**	6(25%)	148(41.1%)	0.473(0.183-1.220)	0.121	0.601(0.192-1.875)	0.38
	No	18(75%)	210(58.9%)	**1**		**1**	
Length of hospital stay	≥72hrs	18(75%)	156(43.3%)	3.92(1.522-10.115)	0.005	3.06(1.11-8.47)	0.031
	<72 hrs	6(25%)	204(56.7%)	**1**		**1**	
Occupational status							
Housewife		2(8.33%)	80(22.22%)	1		1	
Farmer		13(54.17%)	61(16.94%)	0.117(0.026-0.539)	0.006	0.290(0.027-3.071)	0.304
Merchant		4(16.67%)	48(13.33%)	0.300(0.053.1.700)	0.174	0.524(0.064-4.280)	0.54
Others		5(20.83%)	171(47.5%)	0.855(0.162-4.502)	0.853	1.467(0.143-15.1)	0.747

6. DISCUSSION

On human skin and mucosa, *S. aureus* bacteria are a common flora. Most of the time it doesn't do any harm, but occasionally it might lead to illnesses that can be fatal or very mild. Hospitals and the community can become infected with *S. aureus*, especially MRSA. MRSA growth and dissemination make therapy more difficult and present a risk to the control of wound infection (19).

This investigation found that 23.4% of the samples had *S. aureus*. This is comparable to earlier data from four Ethiopian hospitals (21.7%) and Hawassa, Ethiopia (23.2%)(37), and four Hospitals in Ethiopia 21.7%(36). However, it is lower than previous prevalence reports in Ethiopia, including Debre Markos Referral Hospital 29.3%(35), Dessie 34.58%(18), Arbaminch 49.7%(4), and meta-analysis pooled estimate of wound infections due to *S. aureus* 36% (42), and 47.4% in Addis Ababa(33). The variance in the prevalence may result from differences in the study population, duration, and S. aureus detection technique. Higher *S. aureus* prevalence compared to the present finding was also reported from other countries, e.g. two referral hospitals in Asmara, Eritrea 63.1%(32), and Brazil 51.5%(43). These variations may result from the study's methodology, time frame, and the population's socioeconomic standing.

In the present study, 26.7% were MRSA from the *S. aureus* isolates. This is in line with a previous report of 28.3% in Dessie(18), 26.8% in India(30), and four referral hospitals in Ethiopia 24.5%(36). However, it is lower than previous studies in Ethiopia 45.1%(35) and 82.3%(4), 50%(42); and Eritrea 72% (32). However, the MRSA proportion in the current study is higher than the previous study conducted in Hawassa, Ethiopia (8.8%)(37). The prevalence of MRSA from the total participant was 6.3% this is comparable to studies conducted at four hospitals in Ethiopia 5.3%(36), Dessie 9.8%(18), and Brazil 8.7%(43). But much lower than other study in Arbaminch 40.4%(4), Addis Abeba 37.43%(33) and Debre Markos Referral Hospital 13.2%(35), Eritrea 82 (63.1%)(32). But it is greater than the study done in Hawassa (2.03%)(37). The variations in MRSA proportion between the previous studies and the current study might arise due to variations in infection prevention practices, drug prescription, and geographic differences.

Concerning the antimicrobial resistance profile of the isolates in the present study, *S. aureus* isolates showed highest resistance to penicillin (87.8%) followed by Ciprofloxacillin (28.9%), tetracycline (26.7%), azithromycin (22.2%), erythromycin and Trimethoprim/Sulfamethoxazole (13.3% each), gentamicin and clindamycin (8.9% each). All of the isolates were susceptible to linezolid which is in agreement with study done in Hawassa(37). The high resistance to penicillin in our study is comparable with previous studies done in Ethiopia; including Dessie Referal Hospital 84.7%(18), Hawassa University Comprehensive Specialized Hospital 91.2% (37), and Yekatit 12 Hospital 96.1% Ethiopia(33). The current study's 8.9% clindamycin resistance is consistent with a prior study conducted in Ethiopia that found 7.7% resistance to the drug (18). These differences may result from the overuse or improper usage of antibiotics, as well as from likely bacterial mutations and horizontal gene transfer.

In this study, MRSA isolates showed high resistance to Penicillin (100%), tetracycline (84.6%), Ciprofloxacin (84.6), and Azithromycin (75%), but moderate resistance to erythromycin (50%), and Trimethoprim/Sulfamethoxazole (41.7%). In line with the present study, 100% penicillin resistance by MRSA was reported from different parts of the country; i.e. Dessie (18), Hawassa(37), and Addis Ababa(33). A comparable finding of gentamycin resistance by MRSA with our study (33.3%) was reported from Yekatit 12 Hospital in Addis Ababa 38.2%(33), but very lower than study done in Arbaminch which was (94%) for gentamycin. However, the 84.6% resistance to ciprofloxacin documented in the present study is higher compared to the results reported in Dessie Referal Hospital 61.5%(18). Our study's high percentage of antibiotic resistance may have resulted from the widespread usage of antibiotics with no prescription to treat *S. aureus* infections(15).

In the current investigation, a prolonged hospital stay (≥72 hours) was found to be significantly linked with MRSA among the clinical and sociodemographic characteristics evaluated for potential association with MRSA. The likelihood of MRSA increased among patients with hospital stays longer than 72 hours (*P=0.031*, AOR: 3.06, 95% CI: 1.11-8.47 This result is consistent with the earlier Ethiopian investigation that documented a significant association between MRSA and hospital stay ≥72 hours in Debremarkos (*P=0.014*, AOR: 7.402, 95% CI: 1.502–39.49)(35), and in other four hospitals in Ethiopia (*P= 0.000*, AOR = 1.856 (0.688–

5.311))(36). On the other hand, a different study conducted in Hawassa (37), Arbaminch (4), did not reveal a statistically significant association with MRSA.

However, other clinical factors such as prior antibiotic use, comorbidity, hospital admission, and prior wound infection that predisposes to infection, as well as other socio-demographic factors like age, sex, occupation, place of residence, and educational status, did not show a statistically significant association with MRSA infection, which is consistent with research from Hawassa (37), Arbaminch (4), and other studies. This could be caused by the demographic variance under study, cross-contamination between hospital patients, or infection control procedures. Cross-contamination of resistant strain bacteria in healthcare facilities may be the cause of the association between MRSA and extended hospital stays.

6.1 Strengths and Limitations of the Study

This is the first study that explored the prevalence of MRSA using phenotypic methods among patients attending WCSH, in CER, of Ethiopia. For a big hospital with a high rate of nosocomial infections, this study's findings and conclusions are helpful. The antimicrobial policy for regional hospitals and tertiary care facilities, which includes planning the management of hospital infections, treatment regimens, and diagnostic techniques, can be informed by these findings.

The fact that research participants were not selected at random is one of the study's limitations; the prevalence of MRSA and *S. aureus* may not accurately represent prevalence. Further limitations of this study include its shorter study period and single-hospital study setting. In addition, Molecular techniques were not used to identify resistance genes.

7. CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

The research showed a significant proportion of *S. aureus* (23.4%) among patients with wound infections at WCSH and 26.7% MRSA among the *S. aureus* isolates. According to the sensitivity results, every strain of *S. aureus* and every strain of MRSA exhibited 100% sensitivity to linezolid. This shows that linezolid may be a useful treatment for *S. aureus* and MRSA infections in the study population.. On the other hand, both *S. aureus* and MRSA showed high resistance to penicillin. Penicillin resistance is a well-known characteristic of *S. aureus,* and the high resistance observed in this study highlights the limited utility of penicillin in treating *S. aureus* and MRSA infections in this setting. Additionally, the investigation found that all MRSA strains and 31.1% of *S. aureus* strains were multidrug resistant. This indicates that these strains were resistant to several antibiotic classes, which presented a serious problem in treating infections caused by these bacteria. The presence of multidrug-resistant strains emphasizes the importance of implementing appropriate antibiotic stewardship programs and infection control measures to prevent the further spread of resistant bacteria.

The finding that MRSA was substantially linked to hospital stays longer than 72 hours raises the possibility that extended hospital stays could raise the risk of contracting MRSA infections. This emphasizes the necessity of stricter infection control protocols in order to stop MRSA from spreading throughout healthcare institutions, especially for patients who will be hospitalized for a longer period.

7.2 Recommendations

WCSH ought to develop antimicrobial stewardship initiatives and improve their infection prevention and control tactics. To halt the spread of bacteria that are resistant to drugs, like MRSA, It is important to establish guidelines on the use of antibiotics in this context at WCSH. To support the current findings, a longitudinal investigation across many healthcare facilities may be undertaken in the future.

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9. ANNEXES

ANNEX 1. Information sheet

Hello!! Good morning/good afternoon? I am_____. I am gathering data for a study that Nejato Ousman, an Addis Ababa University postgraduate student pursuing an M.Sc. in Medical Microbiology, will be doing. He is investigating the prevalence of methicillin-resistant *Staphylococcus aureus* and its contributing factors in WCSH patients who have wound infections. I will extend an invitation to you to participate in this study. I would like to collect a sample from your wound and ask you some questions about your clinical and socio-demographic details if you allow me. As we go through the questionnaire, if there are any terms that you don't understand, please ask me and I'll take the time to clarify. You are free to choose whether or not to participate; it is entirely voluntary. Nothing will change about the care you receive at this hospital, regardless of your decision to participate or not. You have the option to end the interview at any moment or skip any questions you choose not to answer if you agree to participate. There are no right or incorrect responses. All I want to hear are your thoughts and personal experiences.

Your involvement in this research will assist me in addressing the research questions, but there are no risks, advantages, or financial compensation associated with it. Your provided information will be treated as fully secret, and identifying numbers will be used instead of names. We will not use your name or any other personally identifying information while presenting or publicizing the study's findings. Your responses will be securely stored so that only the researcher can review and analyze them. It will just take 15 to 20 minutes to complete this questionnaire. Nejato Ousman, the primary investigator, can be reached at 0912460038 if you have any questions.

ANNEX 2. Consent form

Participant Code Number_____

I am fully informed about the purpose of the aforementioned study in the language that I can understand. I was aware of the aim of the study, "Prevalence and associated factors of methicillin-resistant *Staphylococcus aureus* among patients at Werabe Comprehensive Specialized Hospital who have wound infections." I have been instructed that there will be very little discomfort involved in the collecting of wound swab samples. The investigator also assured me that all of my personal information, including laboratory results, would be kept private. I was informed that declining to participate in the study or withdrawing from it would not have an impact on my access to medical care now or in the future. I am aware that there are no risks associated with taking part in this study. I've been told that, depending on the lab results, I might directly benefit from the study. Testing without a name will be done, the sample code, and the results in this paper and the other reports will not be recognized by names.

I've either read the material or had it read to me. I've gotten a chance to ask questions, and the responses have been adequate. I have decided to participate in the study.

_____

Signature of the participant

ANNEX 3. English questionnaire

Section I: participants Socio-demographic data			
NO	Questions	Responses	Skip
1	Age of participant in years	1. Years _____ 2. Don't know/ not sure	
2	Sex of participant	1. Male 2. Female	
3	Participants Educational status	1. Not educated 2. Primary (1-8) 3. Secondary (9-12) 4. Collage (10^{+} or 12^{+}) 5. Others _____	
4	Participants Occupational status	1. Government employee 2. Farmer 3. Merchant 4. Student 5. Housewife 6. Daily laborer 7. Others (specify_____)	
5	Participant residence place	1. Urban 2. Rural	
6	Participant's current marital status	1. Married 2. Single 3. widowed 4. Divorced	

Section II: Clinical information			
NO	Questions	Answers	skip
1	Have you been admitted to a hospital in the last 12 months	1. Yes 2. No	
2.	Do you have any co-morbidities (e.g. Cardiac, diabetes, HIV etc.)	1. Yes 2. No	If no skip to question 4
3	If yes what is the disease	_____	
4	Did you have a wound infection in the last 12 months	1. Yes 2. No	
5	In the current visit, how long have you stayed at the hospital	_____	

6	Did you use antibiotics in the last 12 Months	1. Yes 2. No	
7	If yes was it prescribed by a health Professional	1. Yes 2. No	
8	Did you finish the whole dose or did you discontinue using it	1. Yes, I have finished the whole dose 2. No, I discontinued the treatment	
Section III: Wound information and patient status			
NO	Questions	Answers	skip
1	Type of wound	1. Surgical wound 2. Ulcer 3. Burn wound 4. Trauma 5. Others_____	
2	Depth of wound	1. Deep wound 2. Superficial wound	
3	Patient setting in the hospital	1. Inpatient 2. Outpatient	
4	Ward the patient is admitted to	_____ward	

ANNEX 4. Amharic information sheet

ክፍል 1 የመረጃ ቅፅ

እንደ ምን አደሩ/እንደ ምን ዋለ? እኔ _____እባላለሁ።

የአዲስ አበባ ዩኒቨርስቲ የድህረ ምረቃ ተማሪ የሆነው ነጃቶ ዑስማን በሜዲካል ማይክሮባዮሎጂ M.Sc እየተከታተለ የሚሰራውን ጥናት መረጃ እየሰበሰብኩ ነው። ሜቲሲሊን መቋቋም የሚችል ስታፊሎኮከስ ኦሬየስ የተስፋፋበትን ሁኔታና የቁስል ኢንፌክሽን ያለባቸው የደብልዩ ሲ ኤስ ኤች ሕሙማን ለዚህ ችግር አስተዋጽኦ የሚያደርጉ ምክንያቶችን በመመርመር ላይ ናቸው። በዚህ ጥናት እንድትካፈሉ ግብዣ አቀርባለሁ። . ከቁስላችሁ ናሙና ሰብስቤ ስለ ክሊኒካል እና ስለ ማህበረሰብ-ዴሞግራፊ ዝርዝር ጉዳዮችዎ አንዳንድ ጥያቄዎችን ልጠይቅዎት እፈልጋለሁ። በጥያቄው ውስጥ ስናልፍ፣ ያልገባችሁ ቃላት ካሉ እባካችሁ ጠይቁኝና ጊዜ ወስጄ ማብራሪያ እሰጣለሁ።

ለመሳተፍ ወይም ላለመሳተፍ የመምረጥ ነጻነት አለዎት; ሙሉ በሙሉ በፈቃደኝነት የሚደረግ ነው ። በዚህ ሆስፒታል ውስጥ የምታገኙትን እንክብካቤ በተመለከተ ምንም ነገር አይለወጥም ። ቃለ መጠይቁን በማንኛውም ጊዜ ለማቆም ወይም ለመሳተፍ ከተስማማችሁ ላለመመለስ የመረጣችሁትን ማንኛውንም ጥያቄ የማቆም ምርጫ አለዎት። ትክክል ምላሾች ወይም የተሳሳቱ ምላሾች የሉም:: መስማት የምፈልገው ሀሳባችሁን እና የግል ተሞክሮዎቻችሁን ብቻ ነው። በዚህ ምርምር ላይ የእርስዎ ተሳትፎ የምርምር ጥያቄዎችን ለመፍታት ይረዳኛል, ነገር ግን ከዚህ ጋር ተያይዞ ምንም ዓይነት አደጋ, ጥቅሞች, ወይም የገንዘብ ካሳ የሉም . የእርስዎ የቀረቡ መረጃዎች ሙሉ በሙሉ ምስጢር ተደርጎ ይቆጠራል, እና መለያ ቁጥሮች በስሞች ፋንታ ጥቅም ላይ ይውላሉ:: የጥናቱን ግኝቶች በምናቀርብበት ወይም በምናስታውቅበት ጊዜ የእርስዎን ስም ወይም ማንኛውንም የግል መረጃ አንጠቀምም። ምላሾችህ አስተማማኝ በሆነ መንገድ ይከማቻሉ፤ ይህም ተመራማሪው ብቻ ሊመረምራቸውና ሊመረምራቸው ይችላል። ይህን ጥያቄ ለመጨረስ ከ15 እስከ 20 ደቂቃ ብቻ ይፈጃል። ነጃቶ ዑስማን, ዋና መርማሪ, ማንኛውም ጥያቄ ካለዎት 0912460038 ላይ መድረስ ይችላሉ::

ANNEX 5. Amharic consent form

ክፍል 2 የስምምነት ቅፅ

የተሳታፊው ልዩ መለያ ቁጥር _____

ከላይ በተጠቀሰው የጥናት ዓላማ መረዳት በምችልበት ቋንቋ የተሟላ መረጃ አለኝ። "በወራቤ ኮምፕሬንሲቭ ስፔሻላይዝድ ሆስፒታል በሚገኙ ታካሚዎች መካከል ሜቲሲሊን መቋቋም የሚችል ስታፊሎኮከስ ኦሬየስ መስፋፋትና ከዚህ ጋር ተያያዥነት ያላቸው ምክንያቶች" የሚለውን የጥናቱን ዓላማ አውቄ ነበር። የቁስል ናሙናዎችን በመሰብሰብ ረገድ እምብዛም ችግር እንደማይኖር ተነግሮኛል:: በተጨማሪም መርማሪው የቤተ ሙከራ ውጤቶችን ጨምሮ የግል መረጃዎቼ በሙሉ በስውር እንደሚቀመጡ አረጋገጠልኝ ። በጥናቱ ለመካፈል ፈቃደኛ አለመሆኔወይም ከጥናቱ ማፈናቀሌ አሁንም ሆነ ወደፊት የሕክምና ክትትል እንዳገኝ እንደማይነካኝ ተነገረኝ። በዚህ ጥናት መካፈል ምንም ዓይነት አደጋ እንደሌለው አውቃለሁ:: እንደ ቤተ ሙከራው ውጤት ከጥናቱ በቀጥታ ተጠቃሚ ልሆን እችላለሁ ተብያለሁ። ያለ ስም መፈተሽ፣ ናሙናው ኮድ ይደረጋል፣ እናም በዚህ ጋዜጣ እና በሌሎቹ ሪፖርቶች ላይ የሚገኘው ውጤት በስም አይታወቅም። ትምህርቱን አንብቤዋለሁ አሊያም አንብቤዋለሁ። ጥያቄ የመጠየቅ እድል አግኝቻለሁ፣ እናም መልሶቹ በቂ ነበሩ። በጥናቱ ለመሳተፍ ወስኛለሁ::

የተሳታፊው ፊርማ……

የእማኝ ፊርማ …… ቀን……..

Annex . የወላጅ/አሳዳጊ ፈቃደኝነት መጠየቂያ ፎርም

ለተሳታፊዎች መረጃ

ጥናቱ ስለምንድነዉ የሚያተኩረዉ?

ጥናቱ በዋናነት የሚያተኩረዉ ቁስል ካለባቸዉ ታካሚዎች ምን ያህለ መዳኒት በተላመደ ተህዋስ እንደተጠቁ እና ይህንን የሚያስከስቱት ተጓዲኝ ምክንያቶች ምን እንደሆኑ ለማወቅ ነዉ።

ጥናቱ የሚያስከትለዉ ጉዳት አለ?

ለዚህ ጥናት ሳቢያ ምንም አይነት ጉዳት አይደርስበትም። ምናልባት በሽታዉ ከተገኝብዎትና መዲህኒት ከወሰዱ፣ ጥቃቅን የጎንዮሽ ምልክቶች ሊኖሩ ይችላሉ። ነገር ግን ከባድጉዳት አይኖርም።

በጥናቱ ወቅት የህጻኑን መረጃ ሚስጥራዊነት ለመጠበቅ ምን ይደረጋል?

በዚህ ጥናት የሁሉም ፈቃደኛ ተሳፊዎች መረጃ በጥንቃቄ ሚስጥራዊነቱ ይጠበቃል። መረጃዎች ወደሶስተኛ ወገን መተላለፍ ካለባቸዉ፣የተሳታፊዎች ስም የሌለዉ መረጃ ብቻ ይተላለፋል።

ለተጨማሪ መረጃ ማንን ላነጋግር?

ለተጨማሪ መረጃ ካስፈለግዎት እርስዎ ወይም ወላጅዎ በሚከተለዉ ስልክ ቁጥር መደወል ይችላሉ። ነጃቶ ኡስማን 0912460038

የፈቃደኝነት መጠየቂያ ፎረም

ፊርማዎን ከመስቀመጥዎ በፊት መጠየቅ የምፈልጉት ነገር ካለመጠየቅ ይችላሉ። ፊርማዎን ሲያስቀምጡ፣ በጥናቱ ለመሳተፍ ፈቃደኛ ሆነዋል ማለት ነዉ።

ስም ……..ፊርማ…… ቀን……

የመርማሪዉ ፊርማ…… ቀን……..

የእማኝ ፊርማ …… ቀን……..

ANNEX 6. Amharic questionnaire

አማርኛ መጠይቅ

	ምዕራፍ አንድ፡ የተሳታፊው ማህበራዎና አካባቢያዊ ሁኔታዎች		
ተ.ቁ	ጥያቄዎች	መልሶች	እለፈ
1	የተሳታፊው እድሜ በአመት	1._____አመት 2. አላዉቀዉም/ እርግጠኛ አይደለሁም	
2.	የተሳታፊው ፆታ	1.ወንድ 2. ሴት	
3	የተሳታፊው የትምህርት ሁኔታ	1. ያልተማረ/ች 2. አንደኛ ደረጃ (1-8) 3. ሁለተኛ ደረጃ (9-12) 4. ኮልጅ (10^{+} ወይም 12^{+}) 5. ልላ ከሆነ ይጥቀሱ_____	
4	የተሳታፊው የስራ ሁኔታ	1. የመንግስት ተቀጣሪ 2. ገበሬ 3. ነጋዴ 4. ተማሪ 5. የቤት እመቤት 6. የቀን ሰራተኛ 7. ልላ ከሆነ ይጥቀሱ ____	
5	የተሳታፊው የሚኖሩት	1. ከተማ 2. ገጠር	
6	የተሳታፊው የጋብቻ ሁኔታ	1. ያገባች/ ያገባ 2. ያላገባች / ያላገባ 3. ባል የሞተባት/ሚስት የሞተበት 4. የተፋታች	

ምዕራፍ ሁለት፡ የተሳታፊው የህክምና መረጃ			
ተ.ቁ	ጥያቄዎች	መልሶች	ይለፈ
1	ባለፈዉ12 ወር ዉስጥ ታመዉ ሆስፒታል ተኝተዉ ያዉቃሉ	1. አዎ 2. አላዉቅም	
2	ተጓዲኝ በሽታ አለቦት ወይ (ለምሳሌ የልብ በሽታ፣ ስኳር በሽታ፣ ኤች አይ ቪ የመሳሰለት)	1. አዎ 2. የለብኝም	የለብኝም ካለ ወደ 4 ቁጥር ይለፉ
3	አዎ ካለ በሽታዎ ምንድነዉ	_____	
4	ባለፈዉ12 ወር ዉስጥ ቁስል ቆስልቦት/ wound infection/ ያዉቃል	1. አዎ 2. አያዉቅም	
5	በአሁኑ ህክምናዎ በሆስፒታል ዉስጥ ለምን ያህል ጊዜ ቆዩ	_____	
6	ባለፈዉ12 ወር ዉስጥ መዲኒት /antibiotics/ ተጠቅመዉ ያዉቃለ	1. አዎ 2. አልተጠቀምኩም	
7	አዎ ካለ መዲኒቱ በጤና ባለሞያ የታዘዘ ነበር	1. አዎ 2. አይደለም	
8	መዲኒቱን ወስደዉ ጨርሰዉ ነበር ወይስ አቋርጠዋል	1. ጨርሸአለሁ 2. አልጨረስኩም	

ምዕራፍ ሶስት፡ የታካሚዉ እና የቁስለ መረጃ			
NO	ጥያቄዎች	መልሶች	skip
1	የቁስለ አይነት	1. የቀድ ጥገና ቁስል 2.አልድን ያለ ቁስል 3. የቃጠል ቁስል 4. አደጋ 5. ላላ _____	
2	የቁስለ ጥልቀት	1. ጥልቀት ያለዉ ቁስል 2. ከላይ ያለቁስል	
3	ታካሚዉ በሆስፒታል ዉስጥ የሚገኝበት ሁናቴ	1. ተኝቶ ታካሚ 2.ተመላላሽ ታካሚ	
4	ታካሚዉ የሚገኝበት ዋርድ	--------ዋርድ	

ANNEX 6. LABORATORY PROCEDURES

1. Procedure for specimen collection and processing

An expert laboratory technologist gathered the samples, taking extra precautions to prevent skin bacteria from contaminating them. Specimens were collected using an aseptic procedure and sterile materials to avoid microorganism contamination. Swabs were taken before the commencement of antibiotic treatment. A sterile cotton-tipped applicator stick was used to gather a sample from the wound site.

Collection Procedure

1. Compile the required materials.
2. Set up a spotless workspace
3. Describe the process to the client and the reason the swab is being taken
4. Assemble the client
5. Clean your hands with soap and water or an antiseptic hand rub that doesn't contain water.
6. Put on clean gloves.
7. Take off any soiled wound dressing that may be there.
8. Take off the gloves, wash your hands, and then put on new ones.
9. completely clean the area with at least 60 to 120 mL of sterile normal saline or sterile water before taking a culture. Make sure the skin around the incision is also completely cleaned. To remove extra saline or water from the wound surface, use sterile gauze.
10. To proceed with the procedure, there needs to be a visible 1 cm2 region of viable wound bed tissue in the wound
11. For five seconds, rotate the swab's tip over a 1 cm2 patch of viable tissue. To remove fluid from the tissue surrounding the wound, apply enough pressure. Keep the swab away from the wound edge and the skin surrounding the wound. Before swabbing the wound, the swab can be pre-moisturized if the wound surface is dry.
12. Insert the swab into the tube right away, then twist the cap shut. Avoid contacting the swab's surface on the tube opening.
13. Take off your gloves. Keep your hands clean.
14. Write the following information on the requisition and specimen container: client identification, wound kind, date and time of collection

15. Transport the sample to the lab as soon as possible. If delayed store it in the refrigerator at2 - 8^0 C until it can be transported.

2. Laboratory procedure for Gram staining technique

Organisms can be identified using the Gram staining reaction based on their morphology and Gram reaction (Gram positive or Gram negative).

1. Clearly label the slides with the patient's number and the date.

2. Create a smear by equally coating a 15-20 mm diameter region on a slide.

3. Allow the slide to air dry, shielded from dust and flies, after drying the stains.

4. Use heat or a chemical (methanol) to fix the dried smear.

5. Apply crystal violet dye to the fixed smear and let it sit for 30 to 60 seconds.

6. Use clean water to rapidly wash the stains.

7. Turn off all of the water and apply Lugol's iodine to the smear, leaving it on for 30 to 60 seconds.

8. Use fresh water to rinse the iodine off.

9. Use acetone alcohol to quickly (a few seconds) decolorize.

10. Use fresh water to wash right away.

11. For one minute, cover the smear with a neutral red or safranin stain.

12. Use fresh water to wash the discoloration away.

13. Clean the slide's back with a clean towel and set it in a draining rack so the smear may air dry.

14. Use the 40 X objective to evaluate the staining and material distribution, followed by the oil-immersion objective to look for bacteria and cells, to examine the smear under a microscope.

Result Interpretation

Gram-positive bacteria	dark purple
Gram-negative bacteria	pale to dark red

Biochemical tests for gram-positive bacteria

1. Laboratory procedure for Catalase test

Through the use of a catalase test, staphylococci that produce the enzyme catalase can be distinguished from streptococci that do not.

Principle

The breakdown of hydrogen peroxide into oxygen and water is accelerated by catalase. Testing for the production of catalase in an organism involves subjecting it to hydrogen peroxide. The organism releases oxygen bubbles if it produces catalase.

S. aureus, the bacteria that generate the enzyme, is identified using this test.

Procedure

Pour a drop of 3% hydrogen peroxide into a slide

using a sterile wooden stick take the test organism and mix it with the hydrogen peroxide Solution

Look for immediate bubbling

Interpretation:

Active bubbling Positive catalase test

No bubbles Negative catalase test

Positive catalase control: *Staphylococcus* species Negative catalase control: *Streptococcus* species

2. Laboratory procedure for Coagulase Test

This test is used to identify *S. aureus* which produces the enzyme coagulase

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

Slide coagulase test

Place a drop of physiological saline on two separate slides

Emulsify the test organism in each of the drops to make a thick suspension

Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the Organism within 10 seconds

Clumping within 10 seconds *S. aureus*

No clump formation within 10 secs . . . . . No bound Coagulase (confirm with tube coagulase test)

Tube coagulase test

Place 1 ml volumes of the plasma in small tubes.

Emulsify several isolated colonies of test organism in 1 ml of rabbit plasma to give a milky suspension.

Incubate the tube at 35°C in ambient air or a water bath for 4 hours.

Examine at 1, 2, and 4 hours for clot formation by tilting the tube through 90°**.**

Leave negative tubes at room temperature overnight and re-examine

Coagulase Positive: Clot of any size

Coagulase Negative: No clot (plasma remains wholly liquid or shows only a flocculent or ropy precipitate

Controls

Positive Coagulase control: *Staphylococcus aureus*

Negative Coagulase control: *Escherichia coli*

Mannitol Salt Agar

Principles of the Procedure

Mannitol Salt agar due to its content of Peptones and beef extract, which provide vital growth elements including nitrogen, carbon, sulfur, and trace nutrients, make agar a nutritious medium. Bacterial species other than staphylococci are partially or completely inhibited by the 7.5% concentration of sodium chloride. The distinction of Staphylococcal species is aided by mannitol fermentation, as demonstrated by a shift in the phenol red indicator. One solidifying agent is agar.

Procedure

Aseptically streak the sample on the plate

Incubate for 24-48 hours at 37 ^{0}c

After the incubation period, observe the agar for bacterial growth

Also, observe the plate for a change in the color of the agar. The presence of a distinct yellow color indicates fermentation of the mannitol sugar by *S. aureus*. Other *Staphylococci* species will not change the color of the agar.

5. Antimicrobial sensitivity testing (disc diffusion method): Modified Kirby-Bauer technique

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to the edge of the disc.

Preparation of turbidity standard

1. Prepare a 1% v/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mix well. **Caution:** Concentrated sulphuric acid is hygroscopic and highly corrosive, therefore do not mouth the pipette, and never add the water to the acid.

2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of distilled water.

3. Add 0.6 ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mix.

4. Transfer a small volume of the turbid solution to a capped tube or screw-cap bottle of the same type as used for preparing the test and control inocula.

When stored in a well-sealed container in the dark at room temperature (20–28 0C), the standard can be kept for up to 6 months.

Laboratory procedure

1. Emulsify several colonies of similar appearance to test organisms in a small volume of Nutrientbroth.
2. Match the turbidity of the suspension against the turbidity of 0.5 McFarland standard
3. With a sterile swab take a sample from the suspension (squeeze the swab against the

side ofthe test tube to remove the excess fluid).

4. Spread the inoculum evenly over the Muller Hinton agar plate with the swab.
5. Using a sterile forceps, place the antimicrobial discs on the inoculated plate.
6. Incubate the plate aerobically at 35-37 ^{0}c for 16-18 hours.
7. Read the tests after checking that the bacterial growth of the test organism is neither tooheavy nor too light.
8. Measure the radius of the inhibition zone. Interpret the reaction of the test organism to eachantibiotic used as sensitive, intermediate, or resistant as per the standard

Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an MSc degree at Addis Ababa University.

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Date: _____

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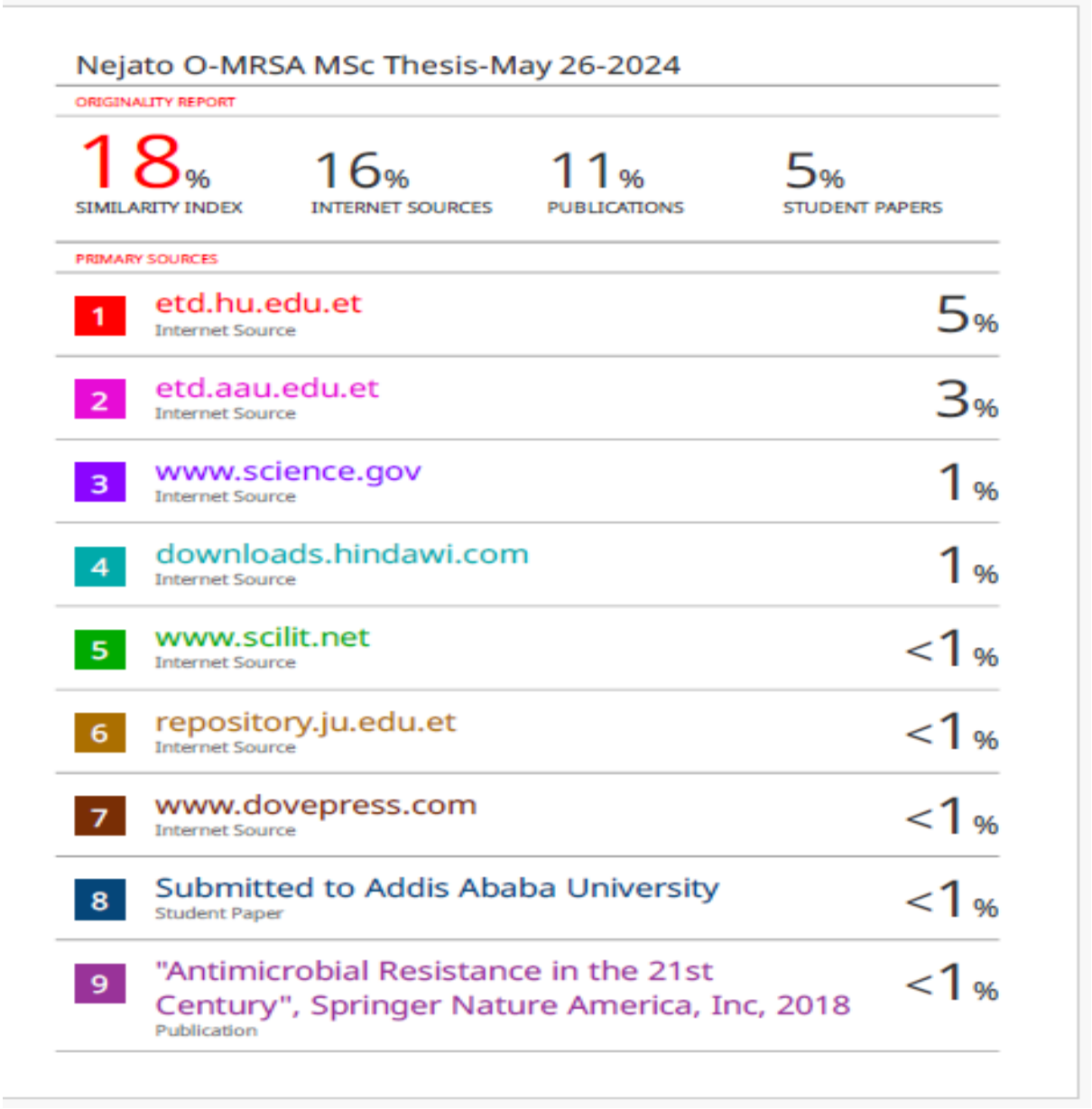
   Date: _____

   Signature: _____

2. Habtamu Biazin (MSc, Ph.D candidate)

   Date: _____

   Signature: _____



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