ADDIS ABABA UNIVERSITY

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DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

PREVALENCEOF NASAL CARRIAGE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*AMONG ADULT HIV-INFECTED AND HIV-UNINFECTED INDIVIDUALS AT ADAMA HOSPITAL MEDICAL COLLEGE, ADAMA, ETHIOPIA

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List of acronyms and Abbreviations

ART	Antiretroviral Therapy
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
CA-MRSA	Community associated MRSA
CLSI	Clinical and Laboratory Standard Institute
CSA	Central Statistical Agency
HA-MRSA	Healthcare-associated MRSA
HIV	Human immune-deficiency Viruses
LA-MRSA	Livestock-associated MRSA
MDR	Multi drug resistant
MHA	Muller Hinton Agar
MRSA	Methicillin resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin susceptible S. *aureus*
PBPs	Penicillin binding proteins
SCC	Staphylococcal cassette chromosome
SOP	Standard Operating Procedures

Abstract

Background: The control of methicillin resistant *Staphylococcus aureus* (MRSA) has become challenging, particularly in immunosuppressed individuals such as HIV-infected patients. Although a global health concern, the data regarding the prevalence and antibiotic resistance pattern of MRSA colonization among HIV- infected and HIV-negative groups is scarce in Ethiopia.

Objective: The main objective of this study was to investigatethe prevalence of nasal carriage and antimicrobial susceptibility pattern of MRSA among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia.

Methods: The study was hospital-based case-control and involved 140 HIV-infected individuals and an equal number of sex- and age group- matched apparently healthy HIV-negative individualsfrom December, 2020 to March, 2021. Data related to demographic, household and clinical features was collected using a structured questionnaire and nasal swab samples were collected by sterile cotton swab. *S. aureus*isolates were confirmed by colony characteristics and biochemical tests while MRSA was detected using Cefoxitin (30μg) discs by Modified Kirby-Bauer disk diffusion method. The data wereanalyzed using SPSS Version-25 and the values were considered to be significant if $p<0.05$ was obtained.

Results: The prevalence of nasal carriage of *S. aureus* and MRSA strains were 34/140(24.3%) and 15/140(10.7%) respectively among the HIV-infected individuals, and the corresponding values within the control group were 19/140(13.6%) and 5/140(3.6%). In both study groups, the MRSA isolates were resistant to penicillin. The proportion of MRSA isolates that were multi-drug resistant were 7/15(46.7%) and 1/5(20%) in HIV-infected patients and HIV-uninfected groups.

Conclusion: This study showed a high prevalence of *S. aureus* and MRSA nasal carriage rates in HIV-infected patients than in HIV-uninfected groups. Special attention should thus be given to the control of MRSA in people living with HIV/AIDS. In addition, regular surveillance and monitoring should be conducted to effectively control this ‘super bug’ infections in high-risk groups like HIV-infected patients.

Key words: *S.aureus*, MRSA, Nasal Carriage, HIV-infected, HIV-negative.

1. Introduction

1.1 Background

Staphylococcus aureus (*S.aureus*) is a Gram-positive spherical-shaped, found singly/cluster forming and non-spore forming bacteriummostly colonizes the skin and nasal mucosa of healthy individuals. It has intrinsic capability to ferment carbohydrates and produce white to deep yellow coloration on solid culture media. The organisms produce catalase and coagulase enzymes, which usually used for its identification [1].

Although,*S.aureus* is normally carried at several body sites of most healthy individuals, anterior nares of the nose is its ecological niche whereprimary replicate and dispersed to other body parts. Approximately, 20% of healthy adult individuals are estimated to be nasal carriers of *S.aureus*persistently,whereas 30% infrequently colonized with the pathogen. However, 50% are apparently not colonized with *S. aureus*[2,3 and 4].

*S. aureus*is a significant cause of clinical infections ranging from mild infections to severe diseases particularly in young, elderly and immune-compromised individuals [5].The diseases caused by these organisms are treatable by antibiotics. However, inappropriate (overuse and misuse) of antibiotics led to the emergence of multi-drug resistants' (MDRs) strains particularly, Methicillin-resistant *Staphylococcus aureus* (MRSA) [6].

MRSA is a highly contagious strain of *S. aureus*resistance to allbeta-lactam antibiotics including methicillin and complicating the treatment of infections. Normally,cell wall of bacteria contains penicillin-binding proteins (PBPs), which have an enzymatic role in the synthesis of peptidoglycan and have high affinity to beta-lactam antibiotics.However, the resistance of MRSA happens by producing altered PBP2a, similar enzyme withlow affinity to beta-lactam antibiotics. PBP2a is encoded by mecA gene which is located on the large mobile genetic element Staphylococcal chromosomal cassette (SCC) and these elements are differ in size and genetic contentamong different strains of MRSA [7, 8].

The remnant strainsof *S. aureus*that are incapable to resist these antibiotics are collectively referred to as methicillin-susceptible S. *aureus* (MSSA).

Although, considerable numbers of effective antimicrobial agents are available, MRSA remainsleading and increasing cause of various invasive infections[9]. MRSA is characterized ashighly resistant to adverse environmental conditions and various antibiotics which enableto colonize skin and nasal mucosatemporarily or permanently, resulting in complicating its prevention and treatment [10].

MRSA transmitted most frequently through direct person-to-person contact, where people are overcrowded, like in security shelters, health facilities, and refugee camps, or indirectly through interaction with shared items or environmental surfaces [11].

The increasing of MRSA infections have become a public health problem and its control gets serious concern globally. MRSA associated infections because a severe burden in terms of longer hospital stays, prolonged antibiotic administration, and increased treatment costs [12].

Currently, based on possible sources of acquirement and distribution as well their genetic features, and drug resistance behavior, MRSA strains are broadly categorizedintohealthcare-associated (HA-MRSA), community-associated (CA-MRSA) and Livestock associated (LA-MRSA). HA-MRSA has been documentedas a key nosocomial pathogensince the 1960s, a year after Methicillin was hosted to combat the resistance of the organism to penicillin [13].

However, CA-MRSA was emerged in the late 1990s as an infection among patients lacking healthcare exposure and rapidly turned into a worldwide threat. Recently, it is increasingly described in numerous groups with confined populations or direct physical contact, including football teams, military trainees, and children in daycare centers, injection drug users, and inmates in correctional facilities [14].

HA-MRSA and CA-MRSA strains isolates have been found to be genetically distinct, implying that HA-MRSA tends to harbor SCC*mec* type I-III, which is larger and therefore likely less transmissible than the types carried by CA- MRSA (SCC*mec* type IV, V, VII. CA-MRSA strains aremore likely to produce virulence factors, most notably Panton-Valentine leukocidin (PVL)

and have greater pathogenic potential than HA-MRSA, but they are commonly sensitive to more non–β-lactam antibiotics [15].

The infections caused by HA-MRSA and CA-MRSA are also different. The community-associated is most frequently infectsthe skin and soft tissue, while hospital-associated pathogen mostly infects the respiratory tract, blood-stream, surgical site, and urinary tract [16]. The other strains of the pathogen adapted to livestock (such as pigs, cattle, and chicken) are called livestock-associated MRSA (LA-MRSA) and are genotypically different from HA-MRSA and CA-MRSA [17].

Table 1: The main characteristics HA-MRSA and CA-MRSA strains

Characteristics	**HA-MRSA**	**CA-MRSA**	**LA-MRSA**
Risk groups	Facility residents, diabetics, dialysis patients, prolonged hospitalizations, ICU patients, IV line	Children, prisoners, athletes, soldiers and etc	Direct contact with agricultural work(Farmers, veterinarians, abattoir employees)
Associated clinical manifestation	Post-operative wound infection, osteomyelitis, pneumonia	Chronic skin infection, abscesses, pneumonia, fasciitis	Wound infection, respiratory associated pneumonia
Molecular markers	*SCCmec* I,II,III	SCC*mec* IV, V, VII	Can arry any of SCC types
PVL toxin	Rare	Common	Rare

Although the effect of MRSA continues to grow in both hospital and Community-associated settings, its infections or colonization rates can be different based on the variety health care facility, the geographical locationand nature of population under study [18].

1.2 Statement of the Problem

MRSA strains were firstidentified in the United Kingdom as a superbug in 1961 within a year after methicillin antibiotic was clinically introduced to combat the resistance of the organism to penicillin. Over the next decade, MRSA isolates were recovered from other European countries, and later from Japan, United States and Australia [19].

The data from 31 European countries showed over 27,000 episodes of MRSA blood stream infections, which resulted in more than five thousand deaths, and more than two hundred and fifty days of hospitalization [20]. In the United States, over 94,000 new invasive MRSA infections are estimated to occur annually, resulting in more than 18,000 deaths. Hospitalization for infections of MRSA cost $14,000, as compared to that stayed for all other diseases ($7,600), recording double the duration of hospitalization [21].

Currently, MRSA becomeburning research area in most developed countries due to its significantburden on patients in all health facility and overcrowded areas. HIV-infected individuals are now recognized as a risk groups for MRSA colonization and infections. Although, the reason for the higher colonization rates is unclear, some factors such as frequent contact with persons in healthcare and community related settings, and frequent exposure to antibiotics, could be included in leading to a greater likelihood of becoming colonized with MRSA [22].

High carriage prevalence of MRSA,up to 16% among HIV-infected individuals reported from different geographical areas indicates the existence of significant associations between MRSA colonization and a higher risk of subsequent infections [23, 24]. Generally, existing literatures shows that, HIV- infected patients are 18-fold increased risk of acquiring MRSA and develop bacteremia and endocarditis than the general population [25]. MRSA-colonized individuals could potentially disseminate the pathogen to other individuals and being colonized is a significant predictor of higher morbidity and mortality. Therefore, accurate detection of MRSA is very important to confirm effective treatment for the affected patient and to avoid further spreading [26].

The first WHO Global report on antibiotic resistance published in 2014 noted the existence of a major gap in monitoring and tracking antibiotic resistance in the African region [27]. Studies

across African countries indicate a varied prevalence of MRSA with higher a prevalence of 29.6% in Nigeria, 27.7% in Kenya, 21.3% in Cameron, 16.8% in Côte d'Ivoire, 14.4% in Morocco, and 12.5% in Senegal [28].

In Ethiopia, there are many studies on the prevalence and antimicrobial susceptibility patterns of MRSA among different study subjects that include schoolchildren, health care workers, Janitors and prisoners and there is also only limited data about the prevalence of MRSA among HIV-infected individuals, however, as to our knowledge, there is no data which indicate the prevalence and antimicrobial susceptibility patterns of MRSA in HIV-infected and HIV-uninfected individual. Therefore, this study was intended to fill the stated gap by determining the prevalence of nasal carriage and describing antimicrobial susceptibility patterns of MRSA among HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia.

1.3 Significance of the study

Along with the only limited availability of published data in the nation, which give the emphasis to be pinpointed on MRSA prevalence in HIV-infected individual and their risk factors, without considering and comparing with HIV-uninfected counterparts. Therefore, this study was helped to address the existing knowledge gap.

Besides, knowledge regarding the magnitude and antimicrobial susceptibility pattern of MRSA in HIV infected individual is essential for health policy maker to develop effective prevention and control strategies.

Lastly, the present study will serve as a base for further studies since studies conducted so far on the same topic are scant.

2. Literature Review

2.1 Epidemiology of MRSA

MRSA infection frequently continues to spread globally in both hospital and community associated setting due to its natural ability to adapt a changing environment [29]. United States, Canada, Japan & Indonesia are some countries with highest rates. Various studies across the world have reported varying carriage prevalence of MRSA: 12% in USA [30], 14.6% in France, 8.1% in Spain, 7.3% in Italy and 7.1% in Israel [31]. Infection caused by MRSA results in increased mortality and morbidity and imposes high burden in terms of health care service. In America, MRSA associated infection kills nineteen thousand hospitalized patients, which are similar to the number of death due to tuberculosis and viral hepatitis [32].

According to studies conducted in North America, the collective prevalence was estimated to be 8.8% (95% CI, 6.0–12.2). The meta-analyzed study by Fainareti *et al,*2014, in Island indicated the prevalence of MRSA colonization among HIV-infected individuals was estimated to be 6.9% (95% CI, 4.8–9.3). Across European studies (560 individuals), the prevalence was found to be 1.0% (95% CI, .3–2.2), whilecrossways Asian studies (n = 1757), the consistent figure was 5.8% (95% CI, 2.8–9.8). [33]. A study carried out in Turkey on Prevalence and risk factors of MRSA colonization in a diabetic outpatient population showed that, 127 (41.9%) and 30 (9.9%) out of 304 patients were colonized by *S. aureus* and MRSA respectively [34].

In Africa, despite the cultural and geographical diversity, which has a significant impact on the epidemiology of *S.aureus*, research on this pathogen has been largely neglected in the past. Therefore, there is limited data on MRSA in Africa. A multicenter study, which involved five major towns in Africa, reported MRSA prevalence of 15% [35].

A study carried out by Matthew et al., 2013 on the prevalence of MRSA among *S.aureus* isolatesindicated almost the increasing trend in different countries. In Libya, the prevalence of MRSAwas 31% in 2007, whereas in Tunisia, it augmented from 16% to 41% in between 2002–2007. In Botswana, the prevalence varied from 23–44% in years 2000–2007 and the prevalence decreased from 36% in 2006 to 24% during 2007–2011 in South Africa. The prevalence was 45%, 52%, 55% and 39% from 2003–2005, in Algeria, Egypt, Ethiopia and Ivory Coast respectively. The same study indicated that, the prevalence of MRSA was lower than 50% untilit

appears to have increased since 2000 in most of the African countries, except in South Africa [36].

Additionally, most MRSA strains in Africa share the common trait like PVL genes, LukS/F-PV, at rates exceeding those of strains from the rest of the world and make them similar to CA-MRSA strains [37].

More specifically, the study conducted by Scott et al., 2011, in SouthAfrica on the prevalence of MRSA nasal carriage among hospitalized patients with tuberculosis indicated that, the prevalence of MRSA with an admission nasal swab was 21% and more common among patients with history of hospitalization, and among HIV-infected individuals [38].

With regard to Ethiopia, the studies done on MRSA among different study subjects are scarce. Among those, the meta-analysis study conducted by Eshetie et al in 2004-2015 on MRSA indicated the MRSA collective prevalence of 32.5% and the isolates were highly resistance to penicillin (99.1%), ampicillin (98.1%), erythromycin (97.2%), and amoxicillin (97.1%)and in less(5.3%)of resistance to vancomycin [39].

Another study conducted by Malinan et al., 2019 on carriage rates, antibiotic resistance and biofilm formation of MRSA colonization among HIV-patients in Arbaminch city, reported the prevalence rate of 20.8%. The study also showed that, patients in the age group of 30–39 years were found to be more colonized by MRSA [40].

2.2. Risk factors for MRSA colonization

Among Several factors reported to predict nasal carriage colonization of *S. aureus* and MRSA, age, sex, HIV infection, intravenous drug use, hospitalization, previous ICU admission, cellulitis at hospital admission, skin ulcers at hospital admission, chronic hemodialysis, diabetes mellitus, neoplasm, presence of a central venous catheter, active smoking and previous antibacterial therapy are reported commonly [23].

In different reports, in addition to these several risk factors existed for *S. aureus* and MRSA colonization, HIV infection has been reported as an independent determinant of colonization with both *S. aureus* and MRSA. Among HIV-infected individuals, factors like male gender, antimicrobial therapy in hospitalized patients, HIV infection in patients greater than 42 years of age and prior hospitalization are reportedly predispose to *S. aureus* colonization[24].

Similarly, the study done by Seybold et al., 2009 reported that, HIV infection predicts *S. aureus* nasal colonization only in patients above the median age of 42 years, but not in younger patients and further demonstrated that history of hospitalization was not a determinant of *S. aureus* colonization in individuals with HIV infection [41].

Among individual with HIV infection, additional recognized determinants of MRSA colonization include age, lower income, history of pneumonia, lymphoma, prior staphylococcal infection (MSSA or MRSA), skin abscess, not receiving antibiotics, crowding, history of hospitalization of household members for more than twice within twelve months, prior/recent hospitalization, use of street drugs, and incarceration [42].

2.3. MRSA colonization and HIV- infected individuals

As the Studies across different geographical areas have reported high MRSA carriage prevalence of about 16% in HIV-infected individuals, HIV-infected individuals are at a greater risk groups for colonization with MRSA [23, 24, and 43]. Accordingly, HIV infected individuals have 18-fold increased risk of acquiring MRSA infections and remain as reservoirs to transmit to other individuals [26]. Different studies reporting on MRSA nasal colonization among the HIV-infected individuals are mostly from the developed world, but very few of such studies have been carried out in Africa.

Kumar et al., 2013 [44] assessed MRSA colonization in 142 HIV-infected patients on ART center at a tertiary care hospital in Eastern India and reported a nasal carriage prevalence of 36.11% for MRSA. Similarly, Hassanzadeh et al., 2012 [43] reported a 12.8% MRSA nasal carriage prevalence in a group of HIV-infected individuals in Iran and Cenizal *et al.,* 2008 [45] reported a 10.3% MRSA nasal carriage prevalence from HIV-infected ambulatory patients attending the HIV clinic of Parkland Hospital in Texas, USA.

Likewise, a study conducted by Kotpal et al., 2016 [46] among HIV-infected individuals attending a teaching hospital reported *S. aureus* and MRSA nasal carriage prevalence of 44% and 5.98% respectively. Kyaw *et al.,* 2012 [47] reported a 5.1% MRSA nasal carriage prevalence among HIV-infected outpatients in Singapore.

In Uganda, a study conducted by Ssenyonga *et al.,* 2018 [48] among People Living with HIV at Nyenga Hospital, reported 41.84% and 5.02% for *S. aureus* and MRSA nasal carriage prevalence respectively. A study by Donkor *et al.,* 2019 [49] in Ghana reported the carriage prevalence of 44.9% and 5.6% for *S. aureus* and MRSA respectively among the HIV-infected children.

In Nigeria, *S. aureus* and MRSA nasal carriage prevalence of 33% and 16% respectively were reported among the HIV-infected population as opposed to the 21% and 8% in the HIV-uninfected participants. The isolates from the HIV-infected individuals were additionally more resistant to other antimicrobials than were those from those without HIV infection [50].

In a study that Job et al., 2018 [51] conducted among People Living with HIV/AIDS Undertaking Antiretroviral Therapy in a Tertiary Hospital in Port Harcourt, Nigeria, they reported 37.79% and 16.13% respectively for *S. aureus* and MRSA nasal carriage prevalence.

In Ethiopia, a study conducted by Gebremedhn*et al.*, 2016 [52], among HIV patients in Mekelle, Northern Ethiopia, reported the overall prevalence of 32.5% and 2.4% for *S. aureus* and MRSA colonization respectively. Another study conducted by Lemma et al., 2015 [24] on MRSA among HIV Infected Pediatric Patients reportedthe prevalence of 51.5% and 16.8% prevalence of *S. aureus* and MRSA colonization respectively.

2.4. Control of MRSA

Giving careful attention to basic personal hygiene that covers, keeping wounds covered, promptly disposing of wound dressings in the trash can, washing hands frequently, and avoiding the sharing of personal items are the primary strategy recommended for preventing MRSA transmission especially in the family and community setting. Other basic infection control practices that include hand hygiene, environmental cleaning and disinfection, the use of aseptic technique for invasive procedures, and patient and health care worker education regarding infection prevention are the foundation for the prevention of many pathogens including MRSA in health care settings [53, 54].

Generally, hand washing is a single most important factor in preventing the spread of MRSA and key ways through which individuals observe hand hygiene by applying hand sanitizers and/or washing with soap [55]. Healthcare workers exposed to MRSA are obliged to maintain a high level of hand hygiene at the points of entry and exit of a MRSA patient's room so as not to transmit it to non-colonized patients due to their hands being key vectors for nosocomial transmission of MRSA. This happensthrough interaction with patients, their body fluids and/ortheir surroundings, before any aseptic procedure [56].

Besides, Measures such as screening for MRSA carriage, improvement of hygiene, isolation of colonized/infected patient and ward are utilized for controlling MRSA in HIV-positive patients [57].

3. Objectives

3.1 General Objective

The general objective of the current study was to determine the Prevalence of nasal carriage and antimicrobial susceptibility pattern of MRSA among adult HIV- infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of nasal carriage of MRSA isolates among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia.
- To investigate the antimicrobial susceptibility pattern of MRSA isolates among adult HIV- infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia.
- To assess if there is variation between HIV-infected and HIV-uninfected in MRSA nasal carriage

3.3 Hypothesis

The prevalence of MRSA is higher among HIV-infected individual than those who are not infected.

4. Materials and Methods

4.1 Study Area

This study was conducted at Adama Hospital Medical College that is found in Adama City, Oromia region. The city is situated 98 km to the East of Addis Ababa. It is located at 8.54 ^{0}N 39.27^{0}E at an altitude of 1712 meters. It sits between the base of an incline to the west, and the Great Rift Valley to the East. Its annual average temperature and rainfall is 20.5^{o}C and 809 mm respectively. Its population size is 338,940 based on figures from the Central Statistical Agency (CSA) projection in 2016.One governmental teaching referral hospital, eight governmental health centers and eighteen private health facilities are available in the city. There is also one regional Laboratory that receives referred samples from all corners of the region. The laboratory works collaboratively with Adama Hospital Medical College on many service aspects such bacteriological culture and AST.

4.2 Study design and Period

A hospital-based case-control study was conducted from December 2020 to March 2021.

4.3 Population

4.3.1 Source Population

All HIV-infected adultindividuals attending ART and all confirmed HIV-negative individuals (control group) from the voluntary counseling and testing site of Adama Hospital Medical College.

4.3.2 Study Population

Adults HIV-infected individuals consecutively attending ART and HIV-negative confirmed by voluntary counseling and testing site of Adama Hospital Medical College and presented to study area during the study period that fulfilled the inclusion criteria were included.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion Criteria

The inclusion criteria for selection of the HIV-infected participants were:

- HIV-infected individuals attending ART.
- Individual with age ≥ 18 years old

The inclusion criteria for the selection of the control group were:

- HIV-negative individuals
- Apparently healthy
- Those with age ≥ 18 years old

4.4.2 Exclusion Criteria:

- Participants on antibiotic treatment for any bacterial infection during data collection
- Participants with clear nasal infection
- Who were critically ill and unconscious during the study period
- Study participants who had nasal bleeding at the time of data collection
- Who were unable to give socio-demographic information and nasal swab specimen were excluded from both study groups.

4.5 Study Variables

4.5.1 Dependent Variables

- Prevalence of MRSA
- Antimicrobial susceptibility pattern

4.5.2 Independent Variables

- Age
- Sex
- Occupation
- Associated factors

4.6 Sample size and Sampling Technique

4.6.1 Sample Size Calculation

Single proportion formula was used to determine the sample size and calculated by taking the prevalence of MRSA among HIV infected Undertaking ART at the Arbaminch province of Ethiopia, which was 20.8% [50].

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

Description:

n = Minimum sample size

Zα= Confidence level at 95% (1.96)

Zβ=power of study 80% (0.84)

P= Estimated MRSA nasal carriage prevalence among HIV-infected=20.8% (a study done at (Arba Minch City, Ethiopia)

$$n = \frac{(1.96)^2 x 0.208 x 0.792}{(0.05)^2} = 254$$

Adding10% contingency, the final sample size for this study was 280. By using 1:1 ratio, 140 participants were included in each study groups.

4.6.2 Sampling technique

A Convenient sampling technique was used to obtainexpected number of the study participants within the given periodof time.

4.7 Recruitment and Consenting procedure

Data collectors were selected informed and oriented how to collect the dataas per the structured questionnaire. The study participants who agreed to participate in the study were signed an informed written consent after which they were enrolled into a study and the objective of the study as well as any associated harm and advantage was explained to them accordingly. The principal investigator was maintaining a logbook separated from the data collection tools, which contain personal identifiers and the participants. This logbook helped in counter-checking to avoid re-sampling.

4.8 Data Collection

4.8.1 Clinical Data

Clinically, a structured questionnaire was used, in addition to participant's medical records, to collect the demographic data, personal history, insertion of medical devices, antibiotic usage, and habit of hand washing and history of hospitalization. During this, the names and any other personal identifiers were not included in the data collection tools but code was assigned.

4.8.2 Laboratory Methods

4.8.2.1Specimen collection and processing

Nasal swabs specimen were collected from the study participants after obtaining informed consent. The swabs were collected by introducing the prepared sterilized cotton swab into both anterior naresand lightly rotating4-5times both clockwise and anticlockwise before withdrawal. Then, Swabs were immediately transported to Adama Public Health Research and Referral Laboratory Center, Medical Microbiology unit for microbiological analysis and all specimens werekeptin refrigerator until the end of this research project.

4.8.2.2 Isolation and identification of *S.aureus*

All collected swabs specimens were inoculated on to Mannitol Salt Agar (MSA) and incubated at 37^0C aerobically for 24 hours. The golden yellow colonies from the MSA plate were sub-cultured on blood agar incubated at 37°C for 24hours. Finally, the isolates with yellow golden colony on Mannitol salt agar and Blood agar, Gram positive cocci in cluster, catalase and coagulase positive were confirmed as *S.aureus.*

4.8.2.3 Detection of MRSA

Each identified *S.aureus* isolates were screened for cefoxitin resistance by the Kirby-Bauer disk diffusion technique according using Mueller-Hinton agar (MHA) plates. The discs were placed in plates and incubated aerobically at 35°C for 24 hours. After incubation, the zone of inhibition was measured with a ruler andinterpreted as resistant, intermediate and sensitive according to Clinical and Laboratory Standard Institute (CLSI,2020) guidelines. Finally, all isolates resistant to cefoxitin (≤21) were confirmed as MRSA.

4.8.2.4 Antimicrobial susceptibility testing (AST)

The suspension of 3 to 5 pure colony from agar plate of each confirmed isolates were done in normal saline solution. The suspension was mixed well and adjusted at 0.5 McFarland standards. A standardized bacterial suspension was swabbed onto the surface of a MHA plate. Then using the standardized Kirby-Bauer disk diffusion method, filter paper disks impregnated with antibiotics were placed on the agar and incubated at 37 ºC for 24 hours, after which the diameter of zones of inhibitions were measured and interpreted according to the breakpoints of CLSI, 2020.

By referring to the standardized tables compiled by CLSI 2020 guidelines, a qualitative report of susceptible, intermediate or resistant can be obtained. The antibiotic susceptibility testing was performed on the following antibiotic discs; Penicillin (10 µg), Cefoxitin (30 µg), Erythromycin (15µg), Clindamycin (10µg), Trimethoprim–Sulphamethoxazol (1.25/23.75µg), Tetracycline (30µg), Ciprofloxacillin (5µg), Chloramphenicol (30µg), Gentamicin (10µg).*S.aureus* (ATCC-25923) was used as control for the antimicrobial susceptibility pattern.

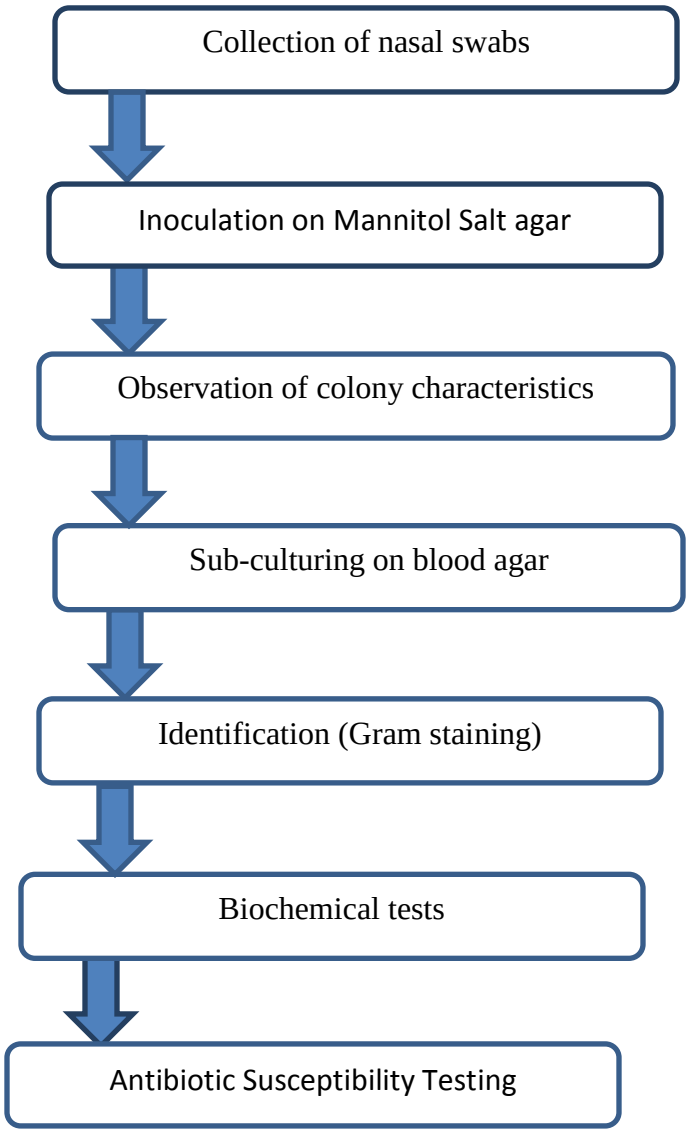


Fig. 1 Chart flowillustrating isolation and identification of *S.aureus*.

4.9 Data Quality Assurance

4.9.1 Pre analytical

Data was collected after the orientation was given to the data collectors. Participants were informed about the purpose of the study when consent wasgiven. The sample collection containers wereappropriately labeled.

The sampleswere collected, transported as quickly as likely and stored according to Standard Operating Procedures (SOPs). All informationregarding study participants collected during the study period was checked for its clearness and completeness regularly. The expiration date of each medium was checked prior to use.

4.9.2 Analytical

Every laboratory tests were performed by well-trained laboratory professionals.The reliability and validity of test result was ensured by the SOP of the host laboratory. Each sampletaken to the testing laboratorywas analyzed as soon as possible. A known strain of *S.aureus* American Type Culture Collection (ATCC 25923) was used to evaluate the performanceof culture media and antibiotic discs.

4.9.3 Post-Analytical

Laboratory Analysis Result Form and logbook were used to manage the result during the study period. Each isolatewas stored in the refrigerator until the end of this research project for any kind of follow-up and future usage.

4.10 Data analysis and interpretation

All collected data were entered and analyzedby using SPSS version 25. The data of study participants in relation to relevant variable were described by using descriptive statistics, frequency and percentage. P-value less than 0.05 were considered as statistically significant and the results were presented in words, tablesandfigures.

4.11 Ethical Consideration

Ethical approval was obtainedfrom Research and Ethics Review Committee of the Department of Medical Microbiology, Immunology and Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University. The Department wrote a letter asking cooperation to Adama Hospital Medical College and Adama Public Health Research and Referral Laboratory Center. Then, permission was obtained from Adama Hospital Medical College and Adama Public Health Research and Referral Laboratory. The data and the sample were collected where the subjects accept the informed consent and sign. Written consent to the study participants was secured before conducting the study and the issue of confidentiality, risk and benefits, the purpose of the study, accountability and academic honesty was maintained throughout the study. In addition to these, participants were informed as they have a full right to refuse or discontinue participating in the study.

4.12 Dissemination of the result

The finding of this study was submitted to Department of Medical Microbiology, Immunology and Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University as MSc thesis. In addition, the finding of this study also submitted to Oromia regional Health bureau, Adama Hospital Medical College and Adama Public Health Research and Referral Laboratory Center and presented to different conferences, stakeholders and other concerned bodies interested to know the magnitude MRSA among HIV infected and non-infected individuals. Finally, it will be submitted to peer reviewed journals for publication.

5. Results

5.1 Socio demographic characteristics

A total of 280 samples were included in the study between December 2020 and March 2021; of which 140 were HIV-infected and the remaining were HIV-negative individuals. The study participant's age in each group of HIV-infected and non-infected ranged between 18 to 71 and 18 to 72 years old, respectively.

The age distribution was similar for each age groups i.e. 18-29 years, 19(13.6%); 30-39 years, 39 (27.9%); 40-49 years, 44(31.4%); 50-59 years, 23(16.4%); 60-69 years, 12 (8.6%) and ≥70 years, 3(2.1%) in both study groups. Males and females comprised 37.1% and 62.9% respectively in both study groups. The majority of study participants 52(37.1%) from HIV-infected had no work and 43(40.7%) from HIV–uninfected were private worker. In both study groups, smaller proportions of the participants were merchants, which 13(9.3%) in HIV-infected and 11(7.9%) among HIV-uninfected groups. Besides, 113(80.7%) of HIV-infected and 127(90.7%) of HIV-uninfected group often washed their hands with soap. (Table 5.1)

Table 2: Socio-demographic and household characteristics of study participants, (HIV-infected and HIV-negative individual at AHMC from December 2020 to March 2021 (n=280).

Socio-demographic characteristics		**HIV-infected**		**HIV-negative**	
		Number	**%**	**Number**	**%**
Age	18-29	19	13.6	19	13.6
	30-39	39	27.9	39	27.9
	40-49	44	31.4	44	31.4
	50-59	23	16.4	23	16.4
	60-69	12	8.6	12	8.6
	≥70	3	2.1	3	2.1
Gender	Male	52	37.1	52	37.1
	Female	88	62.9	88	62.9
Occupation	Private	47	33.6	57	40.7
	Government employee	28	20	29	20.7
	Merchant	13	9.3	11	7.9

	Unemployed	52	37.1	43	30.7
Number of individuals in household	≤5	51	36.4	59	42.1
	5-10	55	39.3	56	40
	≥10	34	24.3	25	17.9
Hand washing with soap	Often	113	80.7	127	90.7
	Rarely	27	19.3	13	9.3

The greater proportions of both study participants 85(60.7%) from HIV-infected and 101(72.1%) from HIV-negative groups had no history of hospitalization. Similarly, 6(4.3%) and 4(2.9%) respectively had history of indwelling medical device and 17(12.1%) and 20(14.3%) respectively used oral antibiotics from HIV-infected and HIV-negative group respectively. However, more proportion of HIV-infected group had a history of underlying disease condition (7.9% vs 3.6%), history of tuberculosis (42.1% vs 21.4) and history of surgical procedure (10.7% vs 9.3%) than the HIV-negative group. (Table 5.2)

Table 3: Clinical Features of study participants, (HIV-infected and HIV-negative individual at AHMC from December 2020 to March 2021 (n=280).

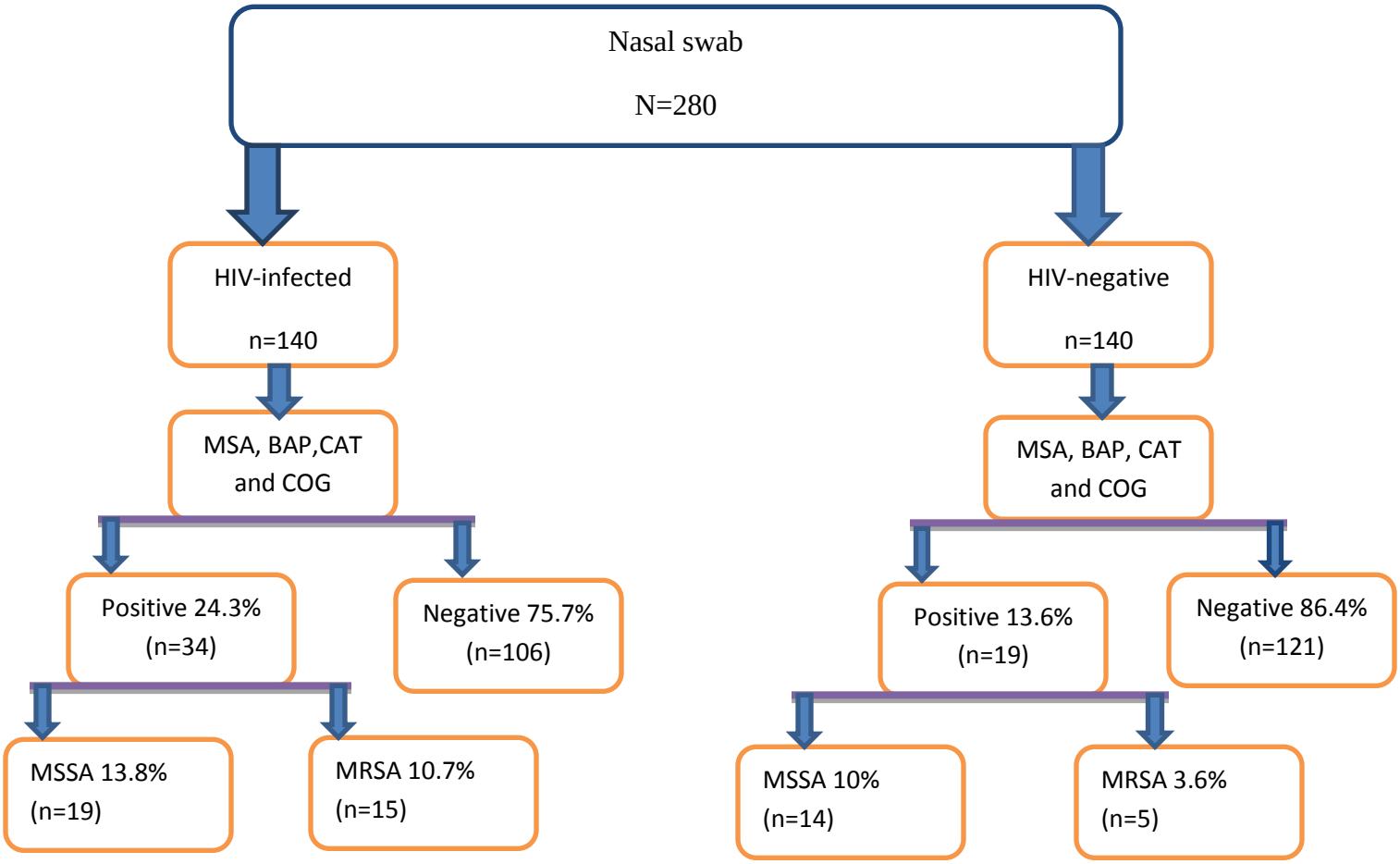
Clinical features		**HIV-infected**		**HIV-negative**	
		Number	%	Number	%
History of hospitalization	Yes	55	39.3	39	27.9
	No	85	60.7	101	72.1
History of IMD	Yes	6	4.3	4	2.9
	No	134	95.7	136	97.1
Usage of oral antibiotics	Yes	17	12.1	20	14.3
	No	123	87.9	120	85.7
Underlying disease condition	Yes	11	7.9	5	3.6
	No	129	92.1	135	96.4
History of TB	Yes	59	42.1	30	21.4
	No	81	57.9	110	78.6
History of	Yes	15	10.7	13	9.3

surgical procedure	No	125	89.3	127	90.7

IMD=Indwelling Medical Device TB= Tuberculosis

5.2 Prevalence of *S.aureus* and MRSA colonization

Out of 280nasal swabs examined for determination of *S. aureus* and MRSA nasal carriage rate, 53 patients were found to be colonized with *S. aureus* among which 34samples were from HIV-infected and 19 from non-infected individuals. The overall prevalence of *S. aureus* and MRSA colonization in HIV-infected was found to be 24.3% and 10.7%, while colonization in HIV-negative was 13.6% and 3.6% respectively. (Fig 5.2)



(Key: n=number of participants, MSA=Mannitol Salt Agar, BAP=Blood Agar Plate, CAT= Catalase, COG=Coagulase)

Figure 5.2: Prevalence results Summary

Out of 140 nasal swabs analyzed from HIV-infected individuals, a high S. *aureus* nasal carriage rate was found among an individuals of age group 30-39 years,12 (35.3%); followed by 40-49 years, eight (20.6%); 50-59 years, six (17.7%); 18-29 years, five (14.7%); 60-69 years, two (5.9%); and ≥70 years, one (2.9%). Similarly, in HIV-negativecounterparts, the high rate of *S. aureus* nasal carriage was found among an individuals of age group 30-39 years with six (31.6%) followed by five (26.3%) in age groups 40-49 years, four (21.1%) in age group 50-59 years and two (10.5%) in age group 18-29 and 60-69 years. However,*S. aureus* was not detected from an individual of age group 70 year or above. *S. aureus* nasal carriage rate was statistically insignificant in relation to age groups of study participants (*p>0.05*).

Among 140 nasal swabs investigated from HIV-infected individuals, *S. aureus* isolates were 34(24.3%), from which 15/52(28.8%) were male while19/88(21.6%) were females. Similarly, out of 19 *S. aureus* isolates from the 140 nasal swabs collected from HIV-uninfected patients; 8/52(15.4%) were isolated from males whereas 11/88(12.5%) were from females. The study reveals that the *S. aureus* carrier percentage was higher among males in HIV-infected as well as in HIV-uninfected individual. *S. aureus* nasal carriage rate was statistically insignificant in relation to gender of study groups (*p>0.05*).

Among the 140 HIV-infected individuals, the growth of *S. aureus* was found in 34 (24.3%), of which 15 (44.1%) were confirmed as MRSA strains and remaining 19 (55.9%) were MSSA. On the other hand, from the 140 HIV-uninfected individuals, 19 (13.6%) *S. aureus* were isolated, five (26.3%) isolates were confirmed as MRSA strain whereas 14(73.7%) were MSSA strains. The results claim higher percentage of MRSA among HIV-infected individuals as compared to HIV-negativeindividual. The association between MRSA isolates and HIV-infected individual was found to be statistically significant.

Of the total 15 (44.1%) MRSA colonization among HIV-infected individual, male accounts five (14.7%) and female accounts 10(29.4%). Whereas, of the total five (26.3%) HIV-uninfected individual colonized with MRSA, two (5.9%) male and three (8.8%) females were colonized with MRSA. More MRSA isolates were isolated from females than males in both study groups. (Table 5.3)

Table 4: Age and sex distribution of *S.aureus* and MRSA among HIV-infected individual at AHMC from December 2020 to March 2021 (n=140).

Demographic feature		***S.aureus* n (%)**	**MRSA n (%)**	**No growth n (%)**	**Total n (%)**
Age	18-29	5 (14.7)	2 (5.9)	14 (73.7)	19(13.6)
	30-39	12 (35.3)	6 (17.7)	29 (70.7)	41 (27.9)
	40-49	8 (20.6)	3 (8.8)	36 (81.8)	44 (31.4)
	50-59	6 (17.7)	2 (5.9)	15 (71.4)	21 (16.4)
	60-69	2 (5.9)	1 (2.9)	10(83.3)	12 (8.6)
	≥70	1 (2.9)	1 (2.9)	2 (66.7)	3 (2.1)
Total		**34 (24.3)**	**15 (44.1)**	**106 (75.7)**	**140 (100)**
Gender					
Male		15 (28.8)	5 (14.7)	37 (71.2)	52 (37.1)
Female		19 (21.6)	10(29.4)	69 (78.4)	88 (62.9)
Total		**34 (24.3)**	**15 (44.1)**	**106 (75.7)**	**140 (100)**

Table 5: Age and sex distribution of *S.aureus* and MRSA among HIV-negative individual at AHMC from December 2020 to March 2021 (n=140).

Demographic feature		***S.aureus* n (%)**	**MRSA n (%)**	**No growth**	**Total n (%)**
Age	18-29	2 (10.5)	0	17 (89.5)	19 (13.6)
	30-39	6(31.6)	2 (10.5)	33 (84.6)	39 (27.9)
	40-49	5 (26.3)	0	39 (88.6)	44 (31.4)
	50-59	4(21.1)	2 (10.5)	19(82.6)	23 (16.4)
	60-69	2 (10.5)	1 (5.3)	10 (83.3)	12 (8.6)
	≥70	0	0	3 (100)	3 (2.1)

Total	**19 (13.6)**	**5 (26.3)**	**121 (86.4)**	**140 (100)**
Gender				
Male	8 (15.4)	2 (10.5)	44 (84.6)	52 (37.1)
Female	11 (12.5)	3 (15.8)	77 (87.5)	88 (62.9)
Total	**19 (13.6)**	**5 (26.3)**	**121 (86.4)**	**140 (100)**

5.3 Antimicrobial susceptibility patterns of the MRSA isolates

This study has shown the presence of the levels of resistance of *S.aureus* to 9 selected antibiotic. All the isolates of *S aureus* from both groups were resistant to penicillin while all those from HIV-negative group were susceptible to Gentamycin.The high proportions of MRSAfrom the HIV-infected cases showed susceptibility to Gentamycin 14(93.3%), Chloramphenicol13(86.7%), Ciprofloxacin 12(80%), Clindamycin 11(73.3%), cotrimoxazole 5(33.3%), Erythromycin 5(33.3%), Tetracycline 5(33.3%).Strains from HIV-negative cases were also susceptible to the above-mentioned antibiotics, but the percentage susceptibility found among these groups was vary, hence the susceptibility profile of Chloramphenicol4(80%), Ciprofloxacin 4(80%), Clindamycin 4(80%), cotrimoxazole 2(40%), Erythromycin 2(40%), and Tetracycline 2(40%). (Table 5.5)

Table 6: Antimicrobial susceptibility pattern of MRSA Isolates among HIV-infected (n=15 and HIV-negative (n=5) individual at AHMC from December 2020 to March 2021.

Antibiotics	**HIV-infected**				**HIV-uninfected**			
	S	**I**	**R**	**R%**	**S**	**I**	**R**	**R%**
Penicillin	0	0	15	100	0	0	5	100
Erythromycin	5	2	8	53.3	3	0	2	40
Clindamycin	11	0	4	26.7	4	0	1	20
Trimethoprim-Sulphamethoxazol	5	3	6	40	3	1	1	20
Tetracycline	5	3	7	46.7	2	1	2	40
Ciprofloxacin	12	1	2	13.3	4	1	0	0

Chloramphenicol	13	0	2	13.3	4	0	1	20
Gentamicin	14	0	1	6.7	5	0	0	0

AHMC= Adama Hospital Medical College S = susceptible; I = intermediate; R = resistant.

5.4 Antibiogram of multi drug resistance MRSA

MRSA isolates were resistant to three to five drugs. Hence, seven of the MRSA isolates (46.7 %) have shown multidrug resistance (MDR) among HIV-infected individualswhereas one MRSA isolate (20%) have shown multi drug resistance from HIV-uninfected counterparts. The highest resistance pattern (13.3%) was observed for three and five antibiotics with pattern of E/TET/COT and E/TET/COT/CLN/CHL respectively. One resistance pattern E/TET/COT/CLN) was observed to for four antibiotics. Similarly, the resistance pattern formed from three antibiotics (E/CPR/GEN) and (E/TET/CPR was observed. In HIV-uninfected individuals, only one resistance pattern of E/TET/COT was observed for three antibiotics.

Table 7: Multi-drug resistance nature of MRSA isolates among study participants at AHMC, Adama, Ethiopia.

HIV-infected		**HIV-uninfected**	
Antibiotics	**Resistant Strains (No (%))**	**Antibiotics**	**Resistant strains (No (%))**
E/TET/COT	2 (13.3)	E/TET/COT	1(20)
E/TET/COT/CLN	1 (6.7)		
E/TET/COT/CLN/CHL	2 (13.3)		
E/CPR/GEN	1 (6.7)		
E/TET//CPR	1 (6.7)		

E=Erythromycin, TET=Tetracycline COT=Co-trimoxazole CLN=Clindamycin, CHL=Chloramphenicol CPR=Ciprofloxacin , GEN=Gentamicin

6. Discussion

To the best of our knowledge, this study is the first of its nature of being case-control study onMRSA nasal carriage among HIV-infected and HIV-negative individuals in Adama Hospital Medical College in particularly and in Ethiopia at large. The findings from this study provides a baseline data pertaining to the prevalence and antimicrobial susceptibility profiles of nasally colonized MRSA among HIV-infected and HIV-negative individuals in Adama Hospital Medical College.

The outbreaks of MRSA infection are very common in the area where people areovercrowded especially in security shelters, health facilities and refugee camps; this strongly supports the concept that the transmission of *S. aureus* is facilitated by close personal contact [11]. Additionally, the rate of nasal carriage mainly depends on various factors includinggeographical area,clinical environment, occupation of patients, and their immune status.

The total of 280 nasal swabs were examined in this study where 140 nasal swabs were from HIV-infected individuals and remaining 140 were HIV-negative individuals. As demonstrated in our study, prevalence of nasal carriage of *S aureus* in the HIV-infected group is more than that in the HIV-negative group, in which nasal swabs from HIV-infected cases yielded a higher carriage rate of 24.3% as compared to HIV-negative group (13.6%). This study indicated the total prevalence rate of nasal carriage of *S. aureus* to be 53/280(18.9%). HIV-infected patients have significantly higher rate ofnasal carriage *S. aureus* than HIV-negative individuals.This high nasal carriage rate of *S. aureus*and development of severe and subsequent infections in this group of people is due to the immunestatus and behavioral features of the individual [46].

Furthermore, the current study revealed a higher rate of *S. aureus* colonization were among patients of age group 30-39 years in both HIV-infected and HIV-negativeindividuals with prevalence rate 12(35.3%) and 6(31.6%), respectively. Similarly, a study carried out by Reinato *et al.,* 2013 have reported 36.1% of *S. aureus* colonization rate among the HIV/AIDS patients of age group 30–39 years and an earlier study conducted at another region of Ethiopia by Manilal *et al*., 2019 reported 45.9% of *S.aureus* colonization among 30-39 age group of HIV-infected individuals[58,40].

With regard to nasal carriage of MRSA, the prevalence of MRSA was found to be higher in HIV-infected (10.7%) than HIV-negative individuals (3.6%). However, the prevalence rate found in this study is considerably lower than that of study conducted in India and Nigeria where the isolation rate was 36.1% and 16.13% respectively[44,51]. Similarly, it was also lower than the study conducted in Arbaminch City, southern Ethiopia among HIV patients of Arba Minch city [40] and among HIV infected pediatric patients in Northwest part of the country [24] where the isolation rate was 20.8% and 16.8% respectively.

Even though, all the mentioned studies showed higher carriage rate as that of ours, none of the studies have compared the carriage rate between both the study groups. Thus, the possible reason for the low prevalence rate observed in the present study can be due to the rare visit of these HIV patients to the health facilities. Moreover, this study was conducted at the time where the study participant stayed at home, frequently hand washing with soap was maintained, sharing of personal items was avoided and using of mask was obliged and recommended as the primary prevention of the emergency of COVID-19. Since the level of hygiene mood of a populace or society could be a factor determining the rate of nasal carriage of MRSA, the mentioned primary prevention strategies commonly applied to prevent the transmission of COVID-19 concurrently limit the transmission of MRSA in both study groups [53, 54].

In contrast, other studies for example, the study by Lemma et al., 2015 [24] was conducted among HIV-infected children which are a unique group for the acquisition of antimicrobial-resistant strains and almost half of the participants of Lemma et al were designated to be at WHO stage III and IV HIV infection, an indication of advanced HIV infection. MRSA prevalence observed among HIV-infected individuals in the current study is consistent with those reported by the studies conducted in Nepal, Iran and Texas where the isolation rate was 13.8%, 12.8% and 10.3% respectively [59, 43 and 45]. However, higher percentage of MRSA among HIV-infected patients was found as compared to non-HIV individuals. Besides, higher carriage rates of *S aureus* was demonstrated in HIV-infected individual with history of hospitalization in the last 6 months, intake of antibiotics in last 3 months, history of surgical procedures, and a history of tuberculosis than their corresponding HIV-negative counterparts with a similar history. Although these carriage rates were more in the HIV-infected group, the difference did not appear to be statistically significant and this comparison does not appear to have been reported by any other researchers.

Of 15 cases HIV-infected individuals from whom MRSA was isolated, 93.3% had history of hospitalization in the last 6 months, 73.3% had history of tuberculosis, 66.7% intake antibiotics in last 3 months and 26.7% had history of surgical procedure.

The antimicrobial susceptibility pattern of MRSA isolates showed 100 % resistance to Penicillin in both HIV-infected and HIV-negative group. The isolates from HIV-infected patients exhibited higher extent of resistance mainly against Erythromycin, Tetracycline and Co-trimoxazole that was similar to the prior studies conducted in Ethiopia among HIV-infected patients [24, 52]. A majority of the MRSA isolates were highly susceptible to clindamycin, chloramphenicol, and Gentamicin [52].

Lastly, seven (46.7%) of the MRSA isolates among HIV-infected and one (20%) amongHIV-uninfected have shown multidrug resistance. This pattern was almost comparable to the study conducted in Mekelle, Ethiopia [52], which reported the prevalence of MDR-MRSA as 50%. However, our finding was indicated higher than that of the study conducted in Arbaminch city, Ethiopia [40] that reported the prevalence of MDR-MRSA as 18.75%. These MDR rates are alarming, though not amazing because, in developing countries like Ethiopia , antimicrobials could be obtained without any prescription, self-medication with antimicrobials is becoming a common practice, many of the consumers do not complete an antimicrobial course and antimicrobial misuse is common[49].

7. Strength and limitation of the study

7.1: Strength

- Towards the best of our understanding, this is the first effort to investigate the prevalence of MRSA in both HIV-infected and HIV-negative patients in the study area and in Ethiopia.
- This study was done at Regional Bacteriology Laboratory, which is well equipped and had access of quality control strains.

7.2: Limitations

- This study was case-control in nature, so does not distinguish between persistent and intermittent carriers.
- As our data were collected conveniently at a single public hospital, it may not reflect all HIV-infected individuals; therefore, consideration of these findings to other populaces should be done with attention.
- This study was unable perform vancomycin sensitivity test by MIC and genetically characterize MRSA isolates due to resource constraints.

8. Conclusion

This study revealed a high prevalence rate in the nasal colonization of *S.aureus* and MRSA in HIV-infected patients than HIV-negative individuals. Besides, MRSA strain from both study groups was found to be highly resistant to penicillin and nearly half were found to be MDR. Special attention should thus be given to the control of MRSA in people living with HIV/AIDS. In addition, regular surveillance and monitoring should be conducted to effectively control this 'super bug' infections in high-risk groups like HIV-infected patients.

9. Recommendation

Based on the findings in this study, the following are recommended:

- Empiric treatment and management of HIV-infected patients should take into account by giving a great concern to MRSA.
- Since the current study was limited to includeother epidemiological risk factors that interplay in colonization of MRSA, additional studies need to be carried out in the study area, with a specific focus on molecular characterization of MRSA strains carried by HIV-infected and uninfected individuals.
- Proper use of antibiotics and applying safety precautions are the basic act to decrease the spread of multidrug resistant strains, particularly MRSA.
- A guidelines should be developed for the prevention and control of MRSA and encourage routine MRSA screening among HIV-infected patients.

10. References

1. Ugwu MC, Mokwe NM, Ejikeugwu PC, Enemor EC, Eze CO, Ugwu BC, Gugu TH. Antibiogram of Staphylococcus aureus from healthy school pupils in Agulu, Southeastern Nigeria. *International Journal of Research*. 2015; 2(4): 5-9.
2. Reid, M.J.A.; Fischer, R.S.B.; Mannathoko, N.; Muthoga, C.; McHugh, E.; Essigmann, H.; Brown, E.L.; Steenhoff, A.P. Prevalence of Staphylococcus aureus nasal carriage in human immunodeficiency virus-infected and uninfected children in Botswana: Prevalence and risk factors. *Am. J. Trop. Med. Hyg*. 2017; 96: 795–801.
3. Muthukrishnan, G.; Lamers, R.P.; Ellis, A.; Paramanandam, V.; Persaud, A.B.; Tafur, S.; Parkinson, C.L.; Cole, A.M. Longitudinal genetic analyses of Staphylococcus aureus nasal carriage dynamics in a diverse population. *BMC Infect. Dis*. 2013, 16, 221.
4. Paulino C, Garcia RD, Ong S. Staphylococcus aureus nasal carriage rates among children between one-to-five years in Barangay Pio Del Pilar, Makati City. *Pediatric Infectious Disease Society of the Philippines Journal*. 2013; 14(1):24-33.
5. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28(3):603–61.
6. Assafi MS, Mohammed RQ, Hussein NR. Nasal carriage rates of Staphylococcus aureus and CA-methicillin resistant *Staphylococcus aureus* among university students. *Int. J. Microbiol*. Res. 2015; 5(4):123-7.
7. Ito T, Okuma K, Ma XX, Yuzawa H and Hiramatsu K. Antibiotic resistance of S.aureus from its whole genome: genomic island SCC. Drug Resist. 2003; 6:41- 52.
8. Srinivasan A, Seifried SE, Zhu L, Srivastava DK, Perkins R, Shenep JL, Bankowski MJ, Hayden RT. Increasing prevalence of nasal and rectal colonization with methicillin- resistant Staphylococcus aureus in children with cancer. Pediatric blood & cancer. 2010 15; 55(7):1317-22.
9. Maranan MC, Moreira B, Boyle – Vavra S, Daum RS. Antimicrobial resistance in Staphylococci. Epidemiology, molecular mechanisms and clinical relevance. Inf. Dis. Clin. N. Am. 1997; 11:813 - 849.
10. Campoccia D, Montanaro L, Arciola CR. The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials. 2006; 27(11):2331-2339.

11. Ringberg H, Cathrine Petersson A, Walder M, Hugo Johansson PJ. The throat: an important site for MRSA colonization. Scandinavian journal of infectious diseases. 2006; 38(10):888-893.
12. Kuehnert MJ, Hill HA, Kupronis BA, Tokars JI, Solomon SL, Jernigan DB. Methicillin resistant Staphylococcus aureus hospitalization in United States. Emerging Infectious Diseases. 2005; 11(6):1472.
13. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA. Methicillin-resistant S. aureus infections among patients in the emergency department. New England Journal of Medicine. 2006; 355(7):666-674.
14. Camargo CH, de Souza MD, Bonesso MF, Da Cunha FP, Barbosa AN, Fortaleza CM. Systemic CA-MRSA infection following trauma during soccer match in inner Brazil: clinical and molecular characterization. Diagnostic microbiology and infectious disease. 2013; 76(3):372-374.
15. Loeffler, A.; Mccarthy, A.; Lloyd, D.H.; Musilová, E.; Pfeiffer, D.U.; Lindsay, J.A. Whole-genome comparison of meticillin-resistant Staphylococcus aureus CC22 SCCmec IV from people and their in-contact pets. Vet. Dermatol. 2013, 24, 538-e128.
16. David M. Z. and Daum R. S., "Community-associated methicillin resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic," Clinical Microbiology Reviews, 2010; 23(3):616-87.
17. Haenni, M.; Saras, E.; Châtre, P.; Médaille, C.; Bes, M.; Madec, J.Y.; Laurent, F. A USA300 variant and other human-related methicillin-resistant Staphylococcus aureus strains infecting cats and dogs in France. J. Antimicrob. Chemother. 2012, 67, 326–329.
18. Santos HB, Machado DP, Camey SA, Kuchenbecker RS, Barth AL, Wagner MB. Prevalence and acquisition of MRSA amongst patients admitted to a tertiary-care hospital in Brazil. BMC Infect Dis. 2010; 10:328.
19. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proceedings of the National Academy of Science USA, 2002; 99(11): 7687-7692.
20. De Kraker ME, Wolkewitz M, Davey PG, Grundmann H. Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to

methicillin-resistant Staphylococcus aureus bloodstream infections. Antimicrobial agents and chemotherapy. 2011;55(4):1598-605.

21. Elixhauser A, Steiner C. Infections with methicillin-resistant Staphylococcus aureus (MRSA) in US hospitals, 1993–2005. In Healthcare Cost and Utilization Project (HCUP) Statistical Briefs [Internet] 2007 Jul. Agency for Healthcare Research and Quality (US).
22. Ramsetty SK, Stuart LL, Blake RT, Parsons CH, Salgado CD. Risks for methicillin-resistant Staphylococcus aureus colonization or infection among patients with HIV infection. HIV medicine. 2010; 11(6):389-94.
23. Farley JE, Hayat MJ, Sacamano PL, Ross T, Carroll K. Prevalence and risk factors for methicillin-resistant Staphylococcus aureus in an HIV-positive cohort. American journal of infection control. 2015; 43(4):329-35.
24. Lemma MT, Zenebe Y, Tulu B, Mekonnen D, Mekonnen Z. Methicillin resistant Staphylococcus aureus among HIV infected pediatric patients in Northwest Ethiopia: carriage rates and antibiotic co-resistance profiles. PLoS One. 2015; 10(9):e0137254.
25. Crum-Cianflone NF, Burgi AA, Hale BR. Increasing rates of community-acquired methicillin-resistant Staphylococcus aureus infections among HIV-infected persons. International journal of STD & AIDS. 2007; 18(8):521-6.
26. Imaz A, Camoez M, Di Yacovo S, Gasch O, Dominguez MA, Vila A, Maso-Serra M, Pujol M, Podzamczer D. Prevalence of methicillin-resistant Staphylococcus aureus colonization in HIV-infected patients in Barcelona, Spain: a cross-sectional study. BMC infectious diseases. 2015; 15(1):243.
27. WHO, WHO's First Global Report on Antibiotic Resistance, News Release, Geneva, Switzerland, 2014.
28. Kesah C, Ben Redjeb S, Odugbemi TO, Boye CS, Dosso M, Ndinya Achola JO, et al. Prevalence of methicillin-resistant Staphylococcus aureus in eight African hospitals and Malta. Clin Microbiol Infect. 2003; 9(2):153-6.
29. Fowler VG, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, Corey GR, Spelman D, Bradley SF, Barsic B, Pappas PA. Staphylococcus aureus endocarditis: a consequence of medical progress. JAMA. 2005; 293(24):3012-21.

30. Rabinowitz RP, Kufera JA, Makley MJ. A hidden reservoir of methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus in patients newly admitted to an acute rehabilitation hospital. 2012; 4(1):18-22.
31. Bilavsky E, Lerman Y, Rabinovich A, Salomon J, Lawrence C, Rossini A, Salvia A, Samso JV, Fierro J, Hochman M, Kazma M. Carriage of methicillin-resistant Staphylococcus aureus on admission to European rehabilitation centres—a prospective study. Clinical Microbiology and Infection. 2012 18(6):E164-9.
32. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. Jama. 2007; 298(15):1763-71.
33. Fainareti NZ, Ioannis MZ, Panayiotis DZ, Josiah DR, and Eleftherios M. Prevalence of and Risk Factors for Methicillin- Resistant Staphylococcus aureus Colonization in HIV Infection. Clinical Infectious Diseases. 2014; 59 (9):1302–11.
34. Kutlu SS, Cevahir N, Akalin S, Akin F, Caylak SD, Bastemir M, Tekin K. Prevalence and risk factors for methicillin-resistant Staphylococcus aureus colonization in a diabetic outpatient population: a prospective cohort study. American journal of infection control. 2012; 40(4):365-8.
35. Breurec S, Fall C, Pouillot R, Boisier P, Brisse S, Diene- Sarr F, Djibo S, Etienne J, Fonkoua MC, Perrier- Gros- Claude JD, Ramarokoto CE. Epidemiology of methicillin- susceptible Staphylococcus aureus lineages in five major African towns: high prevalence of Panton–Valentine leukocidin genes. Clinical Microbiology and Infection. 2011;17(4):633-9.
36. Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP. MRSA in Africa: filling the global map of antimicrobial resistance. PloS one. 2013; 8(7):e68024.
37. Shittu AO, Okon K, Adesida S, Oyedara O, Witte W, Strommenger B, Layer F, Nübel U. Antibiotic resistance and molecular epidemiology of Staphylococcus aureus in Nigeria. BMC microbiology. 2011; 11(1):1-8.
38. Heysell SK, Shenoi SV, Catterick K, Thomas TA, Friedland G. Prevalence of methicillin-resistant Staphylococcus aureus nasal carriage among hospitalised patients with tuberculosis in rural Kwazulu-Natal. South African Medical Journal. 2011; 101(5):332-4.

39. Eshetie S, Tarekegn F, Moges F, Amsalu A, Birhan W. and Huruy K. Methicillin resistant Staphylococcus aureus in Ethiopia: a meta-analysis. BMC Infectious Diseases. 2016; 16(1):1-9.
40. Manilal A, Shewangizaw M, Mama M, Gezmu T, Merdekios B. Methicillin-resistant Staphylococcus aureus colonization in HIV patients of Arba Minch province, Ethiopia: carriage rates, antibiotic resistance, and biofilm formation. Acta Microbiologica et Immunologica Hungarica. 2019; 66 (4):469-83.
41. Seybold U, Supthut-Schröder B, Draenert R, Hogardt M, Bogner JR. Prevalence and risk factors of nasal colonization with Staphylococcus aureus–association with HIV infection in older patients. Scandinavian journal of infectious diseases. 2009; 41(1):63-6.
42. Vieira MT, Marlow MA, Aguiar-Alves F, Pinheiro MG, Freitas Alves MD, Santos Cruz ML, Gaspar S, Curto M, Rocha R, Velarde LG, Araújo Cardoso CA. Living conditions as a driving factor in persistent methicillin-resistant Staphylococcus aureus colonization among HIV-infected youth. The Pediatric infectious disease journal. 2016; 35(10):1126-31.
43. Hassanzadeh, P.; Hassanzadeh, Y.; Mardaneh, J.; Rezal, E.; Motamedifar, M. Isolation of methicillin-resistant Staphylococcus aureus (MRSA) from HIV patients referring to HIV Referral Center, Shiraz, Iran, 2012. Iran. J. Med. Sci. 2015; 40(6):526–530.
44. Kumar S, Bandopadhyay M, Banerjee P, Laskar S. Nasal methicillin-resistant Staphylococcus aureus colonization in HIV-infected patients from eastern India. Saudi Journal for Health Sciences. 2013; 2(1):14.
45. Cenizal MJ, Hardy RD, Anderson M, Katz K, Skiest DJ. Prevalence of and risk factors for methicillin-resistant Staphylococcus aureus (MRSA) nasal colonization in HIV-infected ambulatory patients. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2008; 48(5):567-71.
46. Kotpal R, Bhalla P, Dewan R, Kaur R. Incidence and risk factors of nasal carriage of Staphylococcus aureus in HIV-infected individuals in comparison to HIV-uninfected individuals: a case–control study. Journal of the International Association of Providers of AIDS Care (JIAPAC). 2016; 15(2):141-7.
47. Kyaw WM, Lee LK, Siong WC, Ping AC, Ang B, Leo YS. Prevalence of and risk factors for MRSA colonization in HIV-positive outpatients in Singapore. AIDS research and therapy. 2012; 9(1):1-6.

48. Ssenyonga B, Mwebaze S, Atuhairwe C, MugishaTaremwa I. Perspective For Methicillin-resistant Staphylococcus Aureus Colonization, Antibiotic Susceptibility Patterns and Risk Factors For Colonization Among People Living With HIV At Nyenga Hospital, Buikwe District, in Central Uganda. International Journal of Infection Prevention. 2018; 1(1):1.
49. Donkor ES, Kotey FC, Dayie NT, Duodu S, Tetteh-Quarcoo PB, Osei MM, Tette E. Colonization of HIV-Infected Children with Methicillin-Resistant Staphylococcus aureus. Pathogens. 2019; 8(1):35.
50. Olalekan AO, Schaumburg F, Nurjadi D, Dike AE, Ojurongbe O, Kolawole DO, Kun JF, Zanger P. Clonal expansion accounts for an excess of antimicrobial resistance in Staphylococcus aureus colonising HIV-positive individuals in Lagos, Nigeria. International journal of antimicrobial agents. 2012; 40(3):268-72.
51. Job PA, Alo M, Nwokah EG, Aaron U. Nasal Carriage Prevalence of MRSA in People Living with HIV/AIDS Undertaking Antiretroviral Therapy in a Tertiary Hospital in Port Harcourt. International Journal of Innovative Healthcare Research. 2018; 6(1):12-23.
52. Gebremedhn G, Gebremariam TT, Wasihun AG, Dejene TA, Saravanan M. Prevalence and risk factors of methicillin-resistant Staphylococcus aureus colonization among HIV patients in Mekelle, Northern Ethiopia. Springer Plus. 2016; 5(1):1-9
53. Calfee DP, Salgado CD, Classen D, Arias KM, Podgorny K, Anderson DJ, Burstin H, Coffin SE, Dubberke ER, Fraser V, Gerding DN. Strategies to prevent transmission of methicillin-resistant Staphylococcus aureus in acute care hospitals. Infection Control & Hospital Epidemiology. 2008; 29(S1):S62-80.
54. Smith PW, Bennett G, Bradley S, Drinka P, Lautenbach E, Marx J, Mody L, Nicolle L, Stevenson K. SHEA/APIC guideline: infection prevention and control in the long-term care facility. Infection Control & Hospital Epidemiology. 2008; 29(9):785-814.
55. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, Farr BM. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of Staphylococcus aureus and enterococcus. Infection Control & Hospital Epidemiology. 2003; 24(5):362-86.
56. Sax H, Allegranzi B, Uckay I, Larson E, Boyce J, Pittet D. 'My five moments for hand hygiene': a user-centred design approach to understand, train, monitor and report hand hygiene. Journal of Hospital Infection. 2007; 67(1):9-21.

57. Bischoff WE, Reynolds TM, Sessler CN, Edmond MB, Wenzel RP. Hand washing compliance by health care workers: the impact of introducing an accessible, alcohol-based hand antiseptic. Archives of internal medicine. 2000; 160(7):1017-21.
58. Reinato LA, Pio DP, Lopes LP, Pereira FM, Lopes AE, Gir E. Nasal colonization with Staphylococcus aureus in individuals with HIV/AIDS attended in a Brazilian Teaching Hospital. Revista latino-americana de enfermagem. 2013; 21(6):1235-9.
59. Neupane K, Rayamajhee B, Acharya J, Rijal N, Shrestha D, GC B, Pant MR, Shah PK. Comparison of nasal colonization of methicillin-resistant Staphylococcus aureus in HIV-infected and non-HIV patients attending the National Public Health Laboratory of Central Nepal. Canadian Journal of Infectious Diseases and Medical Microbiology 2018; 2018.

11. Annexes

Annex I:Standard Operative Procedures (SOPs)

1.1 Media Preparation

1. Principle

General purpose, enriched, selective, differential and selective are the general categories of media that are used for growth and cultivation of microorganisms. All types of media used in this facility are prepared in-house. At the time of preparation, each batch of medium is tested with selected organisms to confirm the required growth characteristics, selectivity, enrichment, and biochemical response. Stock reference organisms are maintained in this laboratory for this purpose. A means of labeling and recording the identity of the media, lot and batch numbers, preparation date, expiration date, identity of the preparer, and storage requirements is in place. Since it is onerous to label individual tubes or plates of prepared media, tubes with tightened caps and plated media are stored in bags or boxes that are labeled as described above. All elements of appropriate labeling are recorded and the individual items are traceable to the appropriate data in the record form. Each tube or plate of media is labeled with the identity of the medium and preparation date.

2. Media Preparation: General Guidelines

Wide ranges of culture media are available commercially in the form of dehydrated media. These media are reconstituted by weighing the required quantities and by adding distilled water as per the manufacturer's instructions. When preparing media from dehydrated materials, the manufacturer's instructions should be followed closely, as indicated on the label of the dehydrated media powder. Clean glassware and always use distilled unless specified otherwise. Label containers of dehydrated media used to prepare in-house with the date of receipt date and date first opened. Below are the steps to follow when preparing media.

3. Rehydration

Prior to use, examine the dehydrated material. Caked or discolored media powder should not be used for the preparation of culture media batches. Weigh the appropriate amount of dehydrated medium quickly and accurately. Avoid creating dust. Do not inhale powder. Close the container as soon as possible to prevent contamination. Add half of the required volume of water in the flask (preferably an Erlenmeyer flask that is at least 2-3x the volume of medium), followed by the weighed quantity of powdered media. Agitate briskly for a few minutes until a homogenous

suspension is obtained. Pour the rest of the distilled water down the sides of the vessel to wash any adherent medium back into solution. This is an important step because dry culture media powder above the level of the water may not be sterilized in the autoclave and become a source of contamination. Dissolution is enhanced by allowing agar preparations to stand for five minutes with occasional agitation prior to boiling. Agar-free media will usually dissolve using gentle agitation. Media containing agar should be heated to dissolve the agar before autoclaving. Ingredients are typically mixed on hot plates by placing magnetic stir bars in the bottom of the flask or beaker. Excessive heating should be avoided. When heating is required, gently apply heat and agitate the preparation as required to prevent scorching; however, take care to avoid media eruptions that may occur when agitating a flask of medium that is at or very near the boiling point. Boil as briefly as possible to obtain solution; one minute is usually sufficient. Exposure for longer periods can darken the medium and severely reduce its growth promotion properties. In no case should powdered medium be added to water and immediately placed into an autoclave. Layering and separation of ingredients, precipitate formation and darkening, are likely to occur with diminution of performance.

4. Sterilization

The recommended steam sterilization cycle is 15 minutes at 121°C for 1 liter or less. Larger volumes may require longer cycles. Follow media bottle label directions for length and temperature of sterilization. Do not autoclave media that should not be heat-sterilized. The 49 performance of such media is seriously impaired by subjecting them to heat. For tubed media (except those that require supplements), dispense media before autoclaving.

After sterilization, remove the media from the autoclave as soon as the pressure has fallen to zero. Opening of the autoclave before zero pressure is reached can result in the ejection of media from the sterilization vessels with considerable loss of contents.

5. Adding Enrichments and Supplements

Enrichments and supplements tend to be heat sensitive. Cool the medium to 45-55°C preferably in a water bath before adding enrichments or supplements. Ensure adequate mixing of the basal medium with enrichments or supplements by swirling. Sterile broths may be cooled to room temperature before adding supplements.

Sterile blood used for the preparation of blood agar should be fresh and stored at 2 - 8°C. Blood should be warmed in an incubator (35 - 37°C) before adding to sterile medium. Adequate mixing in a large head-space vessel is essential to ensure aeration of the blood.

Poorly oxygenated blood plates are purplish in color whereas properly aerated blood agar is cherry-red.

6. pH Adjustments

Commercially dehydrated culture media are designed to fall within the specified pH range after steam sterilization (see label of dehydrated media). For filter sterilization, adjust the pH if necessary prior to filtering. Measure the pH at room temperature (25°C). Do not adjust the pH of dehydrated media prior to sterilization. Avoid excessive pH adjustments.

7. Dispensing of Prepared Media

Condensation is minimized if agar is cooled to 50°C prior to pouring (preferably in a water bath). Agar media should be dispensed into 15 x 100 mm or 15 x 150 mm Petri dishes to a uniform depth of 3–4 mm; approximately 20 ml of liquid agar medium will achieve this depth in a 15 x 100 mm plate. Ensure gentle mixing during dispensing and dispense quickly.

After pouring, the plates should be kept at room temperature for several hours to prevent excess condensation from forming on the covers of the dishes. Condensation will also be reduced if plates are stacked so that they cool more slowly.

Note: Covering the agar while it is still hot will allow for the formation of a substantial amount of condensation on the upper lid. If the plates contain condensation, cover them at room temperature for 24 hours to allow the condensation to evaporate. After condensation has evaporated, the plates should be placed in an inverted position and stored in a plastic bag in an inverted position at 4°C.

8. Storage of Prepared Media

Media should always be stored under controlled conditions to ensure its quality through to the expiry date. Label plates with media name and date prepared as soon as they solidify. Allow media to cool at room temperature before storage. Wrap plates in plastic to prevent moisture loss (ten plates per bag) when stored beyond several days. Place a sticker label on the bag indicating the name of the media, batch number, preparation date, expiration date, and storage temperature (2-8 oC). Leave out a certain number of media plates or tubes for sterility testing and QC.

9. Media Preparation Documentation

Record on Media Preparation Record Form

10. Media Expiration Dating

It is good laboratory practice to establish shelf-lives for all prepared media and date-stamp the containers or holders accordingly. The recommended expiration date of prepared culture media varies greatly. The performance and expiration dating of culture media are affected by numerous factors that vary from laboratory to laboratory. For this laboratory:

- Store plated media at 2-8ºC for up to eight weeks (ten plates per bag)
- Store tubed media at 2-8ºC for up to six months (in a box, with caps tight)
- Store sterile NSS and deionized water at room temperature for up to six months (in a box, with caps tight)

Refer to specific media SOPs for storage requirements and expiration dating.

11. Quality Control (QC)

Consistent, documented QC procedures and results are essential for every laboratory. QC tests ensure culture media are prepared according to label directions, and performance characteristics are within specification.

- Perform QC.
- Record results on appropriate QC form.
- C. Label media bag or box with QC label indicating acceptability of QC results.
- Place QC forms in designated location for review and initial by the technologist-in charge. File QC forms in binder after review and signature.

Do not use media with unacceptable QC results for patient testing. Investigate cause of failure and repeat QC testing. Refer to specific media SOPs for appropriate QC procedure. For media prepared in-house, test each batch for pH, sterility, ability to support growth, and ability to produce appropriate biochemical reactions.

pH Value: Check pH of prepared medium (after cooled to 25^{o}C) to ensure the pH falls within the range of the product label. The medium should be discarded if the pH value lies outside the specified range. Testing for sterility: Incubate at least one uninoculated plate from each batch overnight or longer, to verify sterility of the medium. Growth in any of the incubated plates is considered unacceptable result; do not use media batch for patient testing.

Testing for ability to support growth: For selective medium: use at least one strain to test for ability to support growth of the target pathogen; it should also be noted if this strain produces the appropriate biochemical reactions/color on the test medium.

QC procedure: Obtain working control of the selected QC organism. Lightly touch the colony with sterile loop (or needle for butts). Inoculate media appropriately. Do not over-inoculate. Incubate under conditions normally used for media inoculated with clinical specimens.

Interpretation of Results: Nonselective media perform satisfactorily if the QC organisms exhibit adequate growth, expected colony size, and typical colony morphology. Selective media perform satisfactorily if the quality control organisms exhibit adequate growth, expected colony size, typical colony morphology, and inhibition of growth of certain organisms.

12. Inspect media prior to use

Only use media batch with acceptable QC results. In this facility, media with acceptable QC performance are labeled with green stickers on the media bag or box and stored separately. Do not use media lot without these stickers. Refrigerated prepared media should be brought to room temperature prior to inoculation to allow condensation to evaporate or dissipate and to avoid temperature shock to the inoculum. Prior to use, visually check media for proper color, depth, smoothness, hemolysis, excessive bubbles, and contamination. Also check for cracked or damaged plates, and frozen or melted agar. Do not use media that shows any of these signs.

13. References

a. Difco and BBL Manual for Microbiological Culture Media. Maryland, U.S.A., Becton, Dickinson and Company, 2003.

b. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland, 2003.

c. Manual of Clinical Microbiology. American Society for Microbiology (ASM), Washington D.C., USA. 11th edition, 2011.

1.2 Antimicrobial Susceptibility Test Kirby Bauer Method

1. Principle

This procedure describes the standard technique used to determine the in-vitro susceptibility of aerobic nonfastidious organisms. Antimicrobial susceptibility testing (AST) should only be performed with pathogens for which well-standardized methods are available and pathogens whose resistance is known or suspected to be a clinical problem; AST should not be performed on normal flora or colonizing organisms.

Kirby Bauer (KB) is a standardized procedure for performing AST by disk diffusion. A standardized inoculum of the bacteria is swabbed onto the surface of a Mueller Hinton agar (MHA) plate. Filter paper disks impregnated with antimicrobial agents are placed on the agar. After overnight incubation, the diameter of the zone of inhibition around each disk is measured. By referring to the standardized tables compiled by CLSI, a qualitative report of susceptible, intermediate or resistant can be obtained.

2. Materials

a. MHA, Normal Saline Solution (NSS)

b. Antimicrobial disks

c. 0.5 McFarland Standard

d. Sterile cotton swabs

e. Ruler or caliper

3. Specimen

Pure cultures of the organisms from an 18-24 hour agar plate preferably a nonselective medium like sheep blood agar.

1. Quality Control (QC)

Test QC strains by following routine procedure and record results in QC forms.

Record lot number and expiration dates of disks and agar. Compare to expected results based on current CLSI standards. Record any out of control result and proceed with the required corrective action.

4. Procedure

a. Bring agar plates and antibiotic disks to room temperature before use.

b. Prepare bacterial suspension.

The direct colony suspension method is the most convenient method for inoculum preparation. This method can be used for most organisms. Select 3 – 5 well-isolated colonies of the same morphologic type from an agar plate culture. Touch the top of each colony with a loop and transfer the growth into a tube containing 4 – 5 ml of TSB or NSS. Mix well and adjust turbidity with broth or NSS to match 0.5 McFarland standards.

c. Inoculate plate with bacterial suspension

1. Within 15 minutes of adjusting turbidity, dip sterile cotton tipped applicator swab into the inoculum and rotate against the wall of the tube to remove excess inoculum.

2. Swab entire surface of the agar plate three times, rotating plate approximately 60 oC between streaking to ensure even distribution. As a final step, swab the rim of the agar.

3. Allow inoculated plate to stand 3 -15 minutes (no longer than 15 minutes) before applying disks.

d. Apply antibiotic disks to agar surface using sterile forceps or dispenser.

Apply gentle pressure to ensure complete contact of disk with agar. Do not relocate a disk once it has made contact with agar surface. Instead, place a new disk in another location on the agar. Place no more than 12 disks on 150 mm plate and no more than 5 disks on 100 mm plate.

The working supply of antibiotic disks should be stored in a refrigerator (2 – 8 oC) in a tightly capped container with desiccant. Upon removal of the disks from the refrigerator, the package containing the cartridges should be left unopened at room temperature for approximately one hour to allow the temperature to equilibrate; this reduces the amount of condensation on the disks. If a disk dispenser is used, it should have a tight-fitting cover, be stored in the refrigerator, and be allowed to warm to room temperature before use.

e. Invert plate and incubate within 15 minutes of disk application.

Incubate for 16 – 18 hours at 35 ± 2oC in an ambient air incubator

7. Reading and Interpretation

Read plates only if lawn of growth is confluent. If individual colonies are apparent, the inoculum was too light and the test must be repeated.

a. Hold inverted plate a few inches above a black non-reflecting surface. Illuminate plate with reflected light.

b. Use ruler held on the back of the plate to measure the diameter of zone of inhibition.

c. Measure the diameters of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the inhibited growth.

d. Measure the zones to the nearest millimeter (mm).

e. Refer to CLSI M100 tables for interpretation of zone sizes.

6. Reporting

Report the organisms as either Susceptible (S), Intermediate (I), or Resistant (R) to the antimicrobial agents that have been tested.

7. Procedural Notes

a. This method applies to susceptibility testing of non-fastidious organisms; refer to appropriate SOPs for testing for fastidious organisms such as Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, and Neisseria gonorrhoeae.

b. Numerous factors can affect results, including inoculum size, rate of growth, formulation and pH of media, incubation environment and length of incubation, disk content and drug diffusion rate, and measurement of endpoints. Therefore, strict adherence to protocol is required to ensure reliable results.

c. With the exception of staphylococci and enterococci, when reading vancomycin or oxacillin zones, always disregard minute colonies visible only by viewing with transmitted light or by examining with magnifying device.

d. Reading of plates: disregard swarming of Proteus spp. and measure the edge of the obvious inhibition under the veil of swarming.

e. Reading of plates: discrete colonies growing within the inhibition zone may represent a mixed culture or resistant variants: subculture a single colony from the primary plate, re-identify and retest for susceptibility. If the discrete colonies still apparent, measure the colony free inner zone.

f. Emergence of resistance: some bacteria may become resistant during antimicrobial therapy. Performing AST on subsequent isolates after three to four days is recommended.

g. The “Susceptible” category implies that an infection due to the strain may be appropriately treated with the dosage of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

h. The “Intermediate” includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response may be lower than for susceptible

isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and β–lactams in urine) or when a higher than normal dosage of a drug can be used.

i. "Resistant" strains are not inhibited by the usually achievable systemic concentrations of the agent when normal dosage schedules are used; and/or they may have zone diameters that fall within the range where specific, microbial resistance mechanisms are likely and clinical efficacy has not been reliable in treatment studies.

8. References

1) CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. 30th ed. CLSI standard M100 Wayne, PA: Clinical and Laboratory standards Institute; 2018.

2) Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement. CLSI Document M100-S23. Clinical Laboratory Standards Institute (CLSI) 940 Wayne, PA, U.S.A. 2013.

3) Manual of Clinical Microbiology. American Society for Microbiology (ASM), Washington D.C., USA. 10th edition, 2011.

Annex II: Laboratory Result analysis form

Sample Source; **Nasal swab**

Types of culture media used; **MSA**

2.1 Culture observations and work-up on **MSA** QC result: _____

Date	Tech. Initial	Sample ID. NO	Observation and Workup

2.2 Culture observations and work-up on **BPA**

Date	Tech. Initial	Sample ID. NO	Observation and Workup

2.3 Gram Stain; QC Result; Passed Failed

_____

NB; QC Organisms and Expected Results: S. aureus ATCC 25923 – Gram-positive cocci (GPC)

E. coli ATCC 25922 – Gram-negative rod (GNR)

2.4. Biochemical Tests; Catalase; _____

QC date	Lot#	Expiry date	QC results		QC passed/failed
			S.aureus	*S.pyogens*	

QC Organisms and Expected Results: *S. aureus* ATCC 25923 – Positive (bubbles) S. pyogenes ATCC 19615 – Negative (No bubbles)

2.5 Coagulase: _____

QC date	Lot#	Expiry date	QC results		QC passed/failed
			S.aureus	*S.epidermidis*	

QC Organisms and Expected Results: S. aureus ATCC 25923 – Positive (clumping/clotting) S. epidermidis ATCC 12228 – Negative (No clumping/clotting)

2.6 Susceptibility test results

Date Performed:_____ **Tech Initial**:_____			
Antibiotics (Disk abbreviation & content in µg)	**QC Results**	**Test Results**	
	Passed (✓) Filed (x)	Zone Size (mm)	Interpretation(S, I, R)
Penicillin (P-10)			
Cefoxitin (Fox-30)			
Erythromycin (E-15)			
Clindamycin (CM-10)			
Tetracycline (TET-30)			
Ciprofloxacin (CIP-5)			
Chloramphenicol (C-30)			
Gentamicin (GM-10)			
Trimethoprim-sulfamethoxazole (SXT-25)			
Vancomycin (VA-30)			

Test Organism:*S. aureus* 25923 on Mueller Hinton medium. Incubate at 35OC ambient air for 16 – 18 hours.

QC Frequency: Perform QC on each batch number. Refer to AST SOP for test procedure

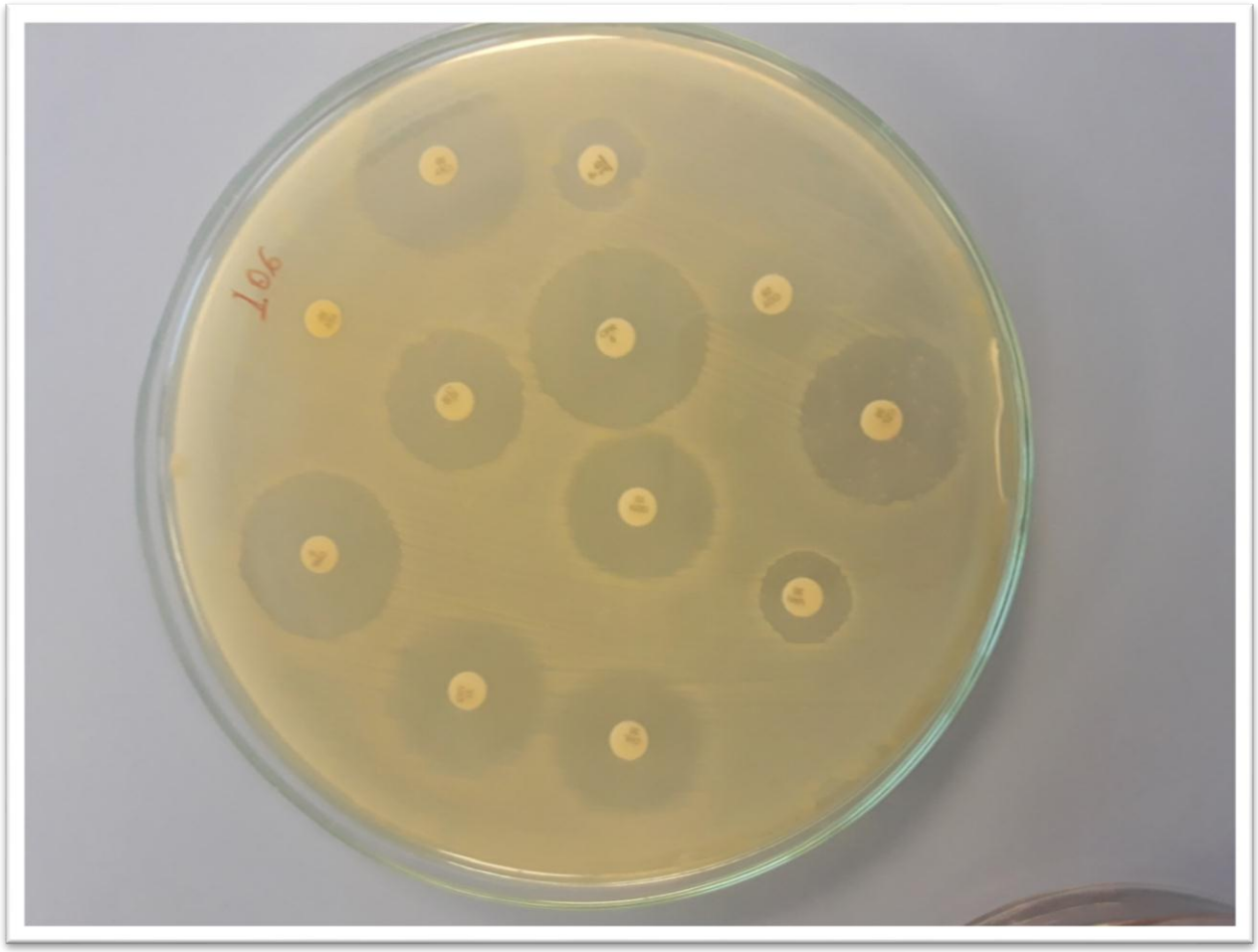
Final report released by: _____Date: _____ Reviewed by: _____ Date: _____

Annex III: Zone of inhibition interpretation chart for Antimicrobials

Antimicrobial Agent	disc content in µg	Interpretive Categories and Zone diameter break points, nearest whole mm		
		S	I	R
Penicillin	10	≥29	-	≤28
Cefoxitin	30	≥22	-	≤21
Erythromycin	15	≥23	14-22	≤13
Clindamycin	2	≥21	15-20	≤14
Tetracycline	30	≥19	15-18	≤14
Ciprofloxacin	5	≥21	16-20	≤15
Chloramphenicol	30	≥18	13-17	≤12
Gentamicin	10	≥15	13-14	≤12
Trimethoprim-sulfamethoxazole	1.25/23.75	≥16	11-15	≤10

Source (CLSI, 2020)

Annex IV: Antibiotic Sensitivity test



Annex V: Participant Information Sheet (English version)

My name is Abdi Negash of Addis Ababa University pursuing a Master's degree in Medical Microbiology. Now I am conducting a study entitled **Prevalence of nasal carriage and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia**

This will be conducted by performing nasal swab of the participating HIV infected individual attending ART and un-infected.

Invitation to participate in the research:

Staphylococcus aureus(*S.aureus*) is a bacterium that is found in the nose andseveral body sites. It can spread to other parts of the body from these sites and cause a wide range of infections in their hosts, and can also spread to others. The infection caused by this *S. aureus* can be treatable by using antibiotics. However, some strains of this bacterium cannot be treated with methicillin because they have acquired resistance to that antimicrobial. Those strains are called methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a worldwide problem in causing a serious and fatal infections in people, particularly, the HIV-infected, because their immune system is less able to fight diseases. In addition, infections caused by MRSA are difficult to treat. It is therefore important to investigate nasal colonization of MRSA in HIV-infected patients in order to contribute information necessary in the management of these patients to promote their health.The collection processof the specimen will involve a routine clinical procedure by qualified personnel.

Expected from participants

As a participant of this study, you are expected to give Nasal Swab. Being asked to give sample does not necessarily mean that you have the infection. When you are confirmed to have this infection, you will be informed by the health care worker and receive proper treatment. However, your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required for participating

You will spend 15-20 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

Risks of participant

Nasal swabs collection will have no effect and you will not get any risk.

Benefits of participation:

By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of this study will provide a baseline data on MRSA prevalence and antimicrobial resistance among HIV-infected patients. This information is necessary for effective management of HIV-infected patients. However, the results will be conveyed to you and found to harbor S. aureus/MRSA; you will be referred to physician for decolonization and/or treatment. You will not be asked to pay any cost of the investigation.

Confidentiality:

All collected materials from you will be coded using numbers and letter. The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access to the information. The information will be encoded in a computer and saved with password protection.

Rights of participants:

If you have read this form, or the content herein has been clearly explained to you, and you have agreed to participate in this study, please note that participation is voluntary and you have the right to withdraw your consent or discontinue participation in the study at any time without penalty. It is also your right to refuse to answer questions you are not comfortable with.Refusal to participate will not result in loss of medical care provided or any other benefits.

Communication:

In case of any questions, problem, unclear ideas and doubt relating to the study, please direct them to:

Abdi Negash (BSc, MSc Student), the principal Investigator of this study on: +251922338501.

Email: abdiinegaa430@gmail.com

For additional information, please contact Dr. Solomon Gebre-Selassie of theDepartment of Medical Microbiology, Immunology and Parasitology on +251911199637

Email: solomongst@yaho.com

P.O.Box: 21656/1000

Annex VI: InformedConsent Form (English Version)

I……, after reading the consent explanation and having been explained to by Abdi Negash (The Principal Investigator), I do voluntarily agree to take part in this research study on **"Prevalence of nasal carriage and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia."** I am aware results of the study may not benefit directly but it might benefit the future effective management of HIV-infected patients.

- I understand that being participation on this study is voluntary; confidentiality of my personal information is guaranteed.
- I understand that at any time I have the right to withdraw from this study without giving a reason.
- The interviewer explains for me, as there is no any risk or discomfort, and extra treatment.
- I understand that as information collected from me are confidential, and they will be reported with my approval and the information will be reported are only the result without my personal information.
- I know there is no extravagant to me without time taken for interview, counseling and health education.

I understand all the information given above and I agreed to participate in this study by my full Interest.

Signature: ----------

Date: ----------/--------/----------

Annex VII: Informed Consent Form (Amharic Version)

እኔ_____ፎርሙንበሚገባካነበብኩት/ተበበልኝቦሃላየጥናትርእስ

“**Prevalence of nasal carriage and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia**.”ላይለመሳተፍበራሰፍላጎትተስማምቻለዉ።

- በጥናቱየምላተፈወበፍቃደነዉ።የምስጠወመረጃበጥብቅእንደሚቆመጥተረድቻለዉ።
- በጥናቱመቀጠልካልፈለኩብማንኝሁምስኣትመቋረጥእንደምቸልተረድቻለዉ።
- በጥናቱበመላተፈየሚድረስብኝጉዳትእንደለለጠየቂወኣስረድቶኝል።
- ያለነፍቃድየጥናቱወጠትየግልመረጃዎችንእንድሚያድርግተረድቻለዉ።
- ምንምኣይነትየገንዘብወጪእንድለብኝከጠየቂተረድቻለዉ።

ከላይየተዛረዘሩትመረጃዎችተረድቶለመላተፍየተስማማሁመሆኑንበፍርማዬኣረጋግጣለዉ።

ፍርማ፡_____

ቀን፡ _____

Annex VIII: Informed Consent form (Afaan Oromoo Version)

<u>Unkaa waliigaltee</u>

Ani---------- kanan jedhamu, unkaa waliigaltee kana ergan dubbisee fi ibsi gahaan erga Abdii Negaashiin naaf kennameen booda qo'annoo mata-dureen isaa "**Prevalence of nasal carriage and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia."** jedhu irratti fedhii kiyyaan irratti hirmaachuuf waliigaleera. Firiin qo'annoo kanaas kallattiidhaan akka dhuunfaatti kan faayidaa addaa naaf hin qabaanne ta'ullee gara fuula duraatti dhukkubsataa HIV qabaman kunuunsuudhaaf akka gargaaru hubadheen jira.

- Qorannoo kanarratti hirmaachuun fedhii kootiin fi odeeffannoon dhuunfaa koo iccitiidhaan akka naaf qabamu hubadheera.
- Sababa tokko osoon hin dhiyeessiin yeroon barbaadetti qo'annoo kana adda kutuuf mirga guutuu akkan qabu hubadheera.
- Yeroon qo'annoo kana irratti hirmaadhu miidhaan hoomaayyuu kan narra hin geenye ta'uu fi wal'aansi adda kan hin barbaachisne ta'uu naaf ibsameera.
- Odeeeffannoon ana irraa funaanamu iccitiidhaan akka naaf qabamuu fi kan gabaafamu odeeffannoo dhuunfaa koo osoo hin taane firii qo'annoo sanaa akka ta'e, akkasumas gabaasni isaa fudhatama kan argatu yoon ani mirkaneesse qofa ta'uu isaa hubadheera.
- Yeroo gaafannoof, gorsaa fi barnoota fayyaa irratti kennamuun ala qisaasa'ummaan tokkolle akka hin jirre bareera.

Odeeffannoowwan armaan olitti eraman irraa hubachuudhaan qo'annoo kana irratti hirmaachuudhaaf fedhii koo guutuudhaan irratti waliigaleera.

Mallattoo:----------

Guyyaa:----------

Annex IX: Questionnaires

Questionnaires for the research on**Prevalence of nasal carriage and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among adult HIV-infected and HIV-uninfected individuals at Adama Hospital Medical College, Adama, Ethiopia.**

Instruction:

- The purpose of the questionnaire is to obtain the information for the study purpose only. The obtained information will go a long way in effective management of HIV-infected patients.
- Your responses will be held in total confidence.
- The questionnaire has three sections. Complete all section.

Section I: Socio- Demographic Characteristics of the Study participants

1. Patient code ……..
2. Age ……..
3. Sex ……..
4. Marital Status ……
5. Occupation ……

Section II: Households Characteristics

8. How many people live in your house? ……
9. How many of your households are HIV infected……...
10. How frequently do you wash your hands with soap? Rarely/often

Section III: Clinical presentation and associated factors questions

1. Is there any history of hospitalization in the past 6 months? Yes No
2. Is there any indwelling medical device in the past 1 year? Yes No
3. Oral antibiotic usage in the past 3 months? Yes No
4. Do you have any underlying disease condition? Yes No
5. If your answer to question number 4 is yes, what condition is it?.....
6. Do you have a history of tuberculosis? Yes No
7. Do you have a history of surgical procedures? Yes No
8. Number of times you are visiting ART clinic? _____

Declaration

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

M.Sc. candidate: Abdi Negash (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr. Solomon Gebre-Selassie (MD, MSc, Associate Professor)

Signature: _____

Date: _____

Place of submission: Addis Ababa University, College of Health Science, School of Medicine, Department of Medical Microbiology, Immunology and Parasitology.