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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* COLONIZATION AMONG CLINICALY TUBERCULOSIS SUSPECTED CLIENTS AT ST.PETER SPECIALIZED HOSPITAL ADDIS ABABA ETHIOPIA.

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This is to certify that the thesis prepared by Akele Kubi entitle Prevalence and Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* Colonization Among Clinically Tuberculosis Suspected Clients at St. Peter Specialized hospital Addis Ababa Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviations

AAU	Addis Ababa University
AST	Antibiotics Susceptibility Test
CLSI	Clinical and Laboratory Standards Institute
CA-MRSA	Community Acquired- Methicillin-Resistant *Staphylococcus aureus*
CI	Confidence Interval
DNase	Deoxyribonuclease
EPHI	Ethiopian Public Health Institute
HC-MRSA	Health Care associated-Methicillin-Resistant *Staphylococcus aureus*
HIV	*Human immune-deficiency Viruses*
MDR	Multi-Drug Resistance
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSA	Manitol Salt Agar
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
OR	Odds Ratio
S.aureus	*Staphylococcus aureus*
SCC	Staphylococcal Cassette Chromosome
SOPs	Standard operating procedures
TB	Tuberculosis
UK	United Kingdom

Abstract

Background: The burden of methicillin resistant *S. aureus* is a major public health concern worldwide; which are challenging both for physicians and patients due to limited choice of therapeutic options and increased cost of care. However there is little information regarding the presence and antibiotic resistance pattern of MRSA colonization on the clinically TB suspected clients.

Objective: The aim of this study was to determine the nasal colonization and antimicrobial resistance patterns of MRSA isolates among clinically tuberculosis suspected clients at St. Peter Hospital Addis Ababa, Ethiopia.

Methods: A hospital based cross-sectional study was conducted from April to July, 2018. A total of 281 Nasal swabs were collected, transported and processed using both SBA and MSA. Disk diffusion method was used for antimicrobial susceptibility testing. MRSA was detected using Cefoxitin(30µg) discs and data related with risk factors were gathered using structured questionnaires. Association of risk factors with colonization of S. aureus/MRSA was assessed using univariate and multivariate logistic regression. A p-value <0.05 was taken as statistically significant association. Data entry and statistical analysis were done using SPSS V-23.

Results: Of 110 *S. aureus* isolates, 34(30.1%) were MRSA strains. The main risk factor for nasal colonization of *S. aureus* in the study area was recent hospitalization. The Susceptibility profiles of MRSA isolates were (85.6%) susceptible to Chloramphenicol, Ciprofloxacin (76.5%), Clindamycin (67.6%), Erythromycin (64.7%), Gentamycin (91.2%), Trimethoprim-sulphamethoxazole (67.6%) and Tetracycline (11.8%). All the MRSA isolates were resistant to Penicillin and sensitive to Vancomicin.

Conclusion: High prevalence of *S. aureus*/ MRSA colonization among tuberculosis suspected clients which suggest a need of attention of the problem in tuberculosis suspected clients. Though only recent hospitalization was associated with S. aureus, colonization by MRSA was not associated with any one of the variables. MRSA isolates were highly resistant to penicillin and highly sensitive to Vancomicin. Further studies are needed on public health significance of MRSA at regional and national level.

Keywords: *S.aureus*, MRSA, Nasal colonization, associated risk factors.

1. Introduction

1.1 Background

Staphylococcus aureus (*S. aureus*) is a bacterium that commonly resides inside the nose and on human skin and sometimes on the vagina and foods. It is highly resistant to adverse environmental conditions and it resists drying as well as high sodium chloride concentrations. This enables a probably temporary and even permanent colonization of skin and nasal mucosa (1).

It is cluster forming, facultative aerobic, gram-positive cocci, none motile with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media. They also ferment mannitol turning mannitol Salt Agar (MSA) to yellow (2). The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins, often called enzymes (clumping factor) used for their identification (3).

Transmission is mainly by direct contact through transient carriage from the hands of health care workers, contaminated equipment or with contaminated environmental surfaces. Person-to-person transmission is the usual form of spread and occurs through contact with secretions from infected skin lesions, nasal discharge or spread via hands (4).

S. aureus has outstanding ability to acquire resistance to antibiotics. Benzyl penicillin was no longer effective for treatment of most *S. aureus* infections within 10 years after its introduction for use because of the acquisition of plasmid-encoded β-lactamase. Penicillin resistant *S. aureus* became pandemic throughout the late 1950s and early 1960s. Methicillin is β-lactam antibiotic invented to treat Penicillin resistant *S. aureus*. However, methicillin resistance *S. aureus* (MRSA) was reported 2 years after the antibiotic was introduced in 1961 in the United Kingdom (5).

MRSA is any strain of *S. aureus* that has developed, through the process of natural selection, resistance to large group of antibiotics called beta-lactam antibiotics, which include the penicillin and the cephalosporin. Virtually all MRSA produce an additional penicillin-binding protein, PBP2a, which confers resistance to all currently available b-lactam agents (6).

It is now recognized that coding for methicillin resistance in MRSA is facilitated by the mecA gene, which is located on the staphylococcal cassette chromosome (SCC), a large mobile genetic element which differs in size and genetic composition among different strains of MRSA. The mechanism of resistance in this manner involves changes or defects brought about by mutation on mecA gene which result in modification to penicillin - binding protein 2a (PBP - 2a) product. The outcome of the event is that it renders the organism resistant to β-lactams and other antibiotics with the same target site (7).

Strains unable to resist these antibiotics are classified as methicillin-sensitive *S. aureus* (MSSA). As MRSA has adapted to survive treatment with antibiotics, including methicillin, dicloxacillin, nafcillin, and oxacillin, it may also be referred to as multidrug-resistant *S. aureus*. MRSA is a bacterium responsible for several difficult-to-treat infections in human. In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an important and increasing cause of postsurgical wound infections, some invasive infections such a septicemia (sepsis), pneumonia, osteomyelitis, endocarditis and other soft tissue infections (8).

MRSA is an important bacterial pathogen causing nosocomial and community-onset infections which has shown increasing endemic and epidemic spread in the last four decades (7, 8); while its control has become a serious concern worldwide (9). These MRSA associated infections impose a serous burden in terms of medical and socio-economic costs and cause significant morbidity and mortality (10).

The classification of MRSA strains based on possible sources of acquisition and dissemination (nosocomial versus community), nature of drug - resistance (single versus multiple), as well as genome characteristics like size of genome. MRSA strains were recently classified as two groups based on possible sources of acquisition, namely community associated (CA-MRSA) and healthcare-associated (HA-MRSA). CA-MRSA isolates are usually less resistant than HA-MRSA isolates. While HA-MRSA has been documented since the 1960s, CA-MRSA infections, defined as MRSA infection among patients lacking healthcare exposure, have emerged as a concern more recently and have been increasingly reported (11).

Table 1.1 The main characteristics HA-MRSA and CA-MRSA strains

Characteristic	HA-MRSA	CA-MRSA
Clinical	surgical site infections, invasive	skin infections, rarely invasive
Epidemiology	old, healthcare	young, athletes, drug users, and military
Antibiotic resistance	multi-drug resistant	β-lactam resistant
Molecular markers	SCCmec I-III	SCCmec IV, V

Generally, MRSA colonization is said to occur in individuals who have frequent exposure to healthcare settings and in those with frequent antibiotic usage as well as immune suppression (12, 13). Traditionally, MRSA is associated with health care institutions following its first detection in the UK in the early 1960s (14). Several risk factors have been suggested for the acquisition of nosocomial MRSA. They include previous or excessive antimicrobial therapy, previous hospitalization, surgery, prolonged hospital stay and close proximity to a patient in the hospital who is infected or colonized with MRSA (14). Male-to-male sexual intercourse and housing conditions are also cited as potential risk factors for CA-MRSA (15, 16).

Colonization rates or infections with MRSA vary by geographical location, type of health care facility, and the specific population being studied. Studies have reported prevalence of MRSA carriage on admission ranging from 2.6 to 8% with higher prevalence reported among elderly patients (17-19).

If MRSA is diagnosed, treatment will vary depending on the following factors: type of infection, location of infection, severity of symptoms and antibiotics to which the strain of MRSA responds. And management of MRSA infections may include: pus drainage from lesion culture and susceptibility testing of drained material wound care and hygiene antimicrobial therapy and finally medication options for MRSA skin and soft tissue infections may include: clindamycin, erythromycin tetracycline trimethoprim- sulfamethoxazole and vancomycin (20-21).

1.2 Statement of the Problem

Strains of MRSA were first detected in the United Kingdom (UK) in the early 1960s (14) soon after methicillin antibiotic was introduced for clinical use. During the next decade, the existence of MRSA strains was reported in the United States with prevalence rate of less than 1% (22). Since then, the endemic and epidemic outbreaks of the organism have been reported worldwide (22-24). Presently, hospital of all sizes, other care centers, and increasing number of different population groups at various communities globally are facing the problem of MRSA infections. MRSA infections are a serious global problem, with considerable impact on patients and substantial healthcare costs. (13).

Now days, MRSA is the hottest research area in most developed countries due to increased mortality and morbidity. Reports emanating from different parts of the world revealed increasing rates in the incidence of MRSA and population at risk. It is estimated that MRSA infections within the health care setting alone affected more than 150, 000 patients annually in the European Union, with an additional cost of 380 million Euros (23). In the United States of America, 80,461 invasive MRSA infections and 11,285 related deaths occurred in 2011, and an estimated annual burden of between $1.4 billion and 13.8 billion was attributed to CA- MRSA (24). Similarly, higher isolation rate of MRSA has been reported from most African countries including Nigeria (29.6%), Kenya (27.7%), Cameroon (21.3%), Cote D'Ivoire (16.8%), and Morocco (14.4%) (25). In Ethiopia, 42.8% isolation rate of MRSA was reported from among health workers of Jimma Specialized Hospital (26).

Accurate detection of Methicillin resistance in *S. aureus* is of the greatest importance to ensure effective treatment for the affected patient and to prevent further transmission. Although the association between tuberculosis and MRSA (co morbidity) is an important clinical problem that needs consideration in order to increase the survival time of TB patients, least has been done in the area of especially tuberculosis prevalent countries. Since TB patients are the ones who have long period of hospital stay, it is expected that they are candidates for acquiring MRSA colonization .More over in Ethiopia, as to our knowledge, even if there are many articles on prevalence and antimicrobial susceptibility patterns on different study subjects like HIV patients, school children, health care workers and prisoners, there is no published data that indicates the

prevalence of MRSA and antimicrobial susceptibility patterns among clinically TB suspected clients.

Therefore, the present study tried to determine the magnitude of MRSA among clinically TB suspected clients, and identifies factors associated with MRSA colonization in clinically TB suspected clients and antimicrobial susceptibility testing on *S.aureus* and MRSA isolate at St. Peter Hospital, Addis Ababa, Ethiopia.

1.3 Significance of the study

Since there is no published data in the nation on the prevalence of MRSA colonization among clinically TB suspected clients, the finding of the magnitude of MRSA on TB suspected clients was helped to address the existing knowledge gap and our findings suggest that *S. aureus* and MRSA should be considered as public health importance.

This study identified factors significantly associated with colonization of S. aureus which help us to downgrading the prevalence of the disease and also the study generated data on AST of MRSA which could help to manage our client's correctly.

Knowing the magnitude, associated factors and antimicrobial Susceptibility pattern of MRSA on clinically TB suspected client's is necessary for local public health authorities and the hospital to develop effective prevention strategies and control guideline. Study the prevalence and risk factor of MRSA colonization may lead to better management of clinically TB suspected clients.

Since the investigator has not come across any study done in Ethiopia, the present study provides information regarding the magnitude of MRSA on clinically TB suspected client's at the study area and in Ethiopia.

2. Literature Review

Since the investigator has not come across any study done on colonization rates and antibiotic susceptibility patterns of MRSA among clinically TB suspected clients in the study area and Ethiopia in general, I am just forced in order to review some of related article as I mentioned below.

According to meta-analyzed study in Island by Fainareti NZ et al, on Prevalence of and Risk Factors for MRSA Colonization in HIV Infection: showed that the combined prevalence of MRSA colonization among (655) HIV-positive individuals was estimated to be 6.9% (95% CI, 4.8–9.3). When data from studies conducted exclusively in North America were separately analyzed, the pooled prevalence was estimated to be 8.8% (95% CI, 6.0–12.2). Across European studies (560 individuals), the prevalence was found to be 1.0% (95% CI, .3–2.2), whereas across Asian studies (n = 1757), the corresponding figure was 5.8% (95% CI, 2.8–9.8). History of hospitalization during the previous 12 months and Previous or current incarceration were associated with MRSA colonization (27).

A study carried out in Turkey by Selda SK et al on Prevalence and risk factors for MRSA colonization in a diabetic outpatient population revealed that out of the 304 patients, 127 (41.9%) were colonized with *S.aureus* and 30 (9.9%) were colonized with MRSA. Overall, 23.6% of all *S.aureus* isolates were MRSA (28). Factors independently associated with an increased risk of MRSA colonization included the presence of connective tissue disease and insulin therapy (28).

By this study, methicillin resistance and other antimicrobial susceptibilities were also evaluated by the Kirbye Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines. And the resistance of MRSA to other antibiotics was as follows: 60% to erythromycin, 46.7% to clarithromycin, 36.7% to tetracycline, 23.3% to clindamycin, 20% to gentamicin, 43.3% to trimethoprimsulfamethoxazole, 13.3% to rifampin, and 43.3% to ciprofloxacin. No resistance to vancomycin was detected (28).

Another study in Atlanta, Georgia by Alicia I.et al, on risk factors for colonization with MRSA in Patients admitted to an Urban Hospital: showed that a total of 53 (7.3%) of 726 patients had a

nares culture positive for MRSA, and 119 (16.4%) had a nares culture that was positive for methicillin-susceptible *S.aureus*. Risk factors for MRSA colonization included antibiotic use within 3 months before admission, hospitalization during the past 12 months, diagnosis of skin or soft-tissue infection at admission and HIV infection (29).

A study carried out in Africa by Matthew E.F et al in January 2013 on MRSA in Africa: They sought to assess the prevalence of methicillin-resistance among *S.aureus* isolates in Africa. They included articles published in 2005 or later reporting for the prevalence of MRSA among *S.aureus* clinical isolates. Thirty-two studies were included. In Tunisia, the prevalence of MRSA increased from 16% to 41% in between 2002–2007, while in Libya it was 31% in 2007. In South Africa (S.Africa), the prevalence decreased from 36% in 2006 to 24% during 2007–2011. In Botswana, the prevalence varied from 23–44% in years 2000–2007. In Algeria and Egypt, the prevalence was 45% and 52% from 2003–2005, respectively. In Nigeria, the prevalence was higher in the northern part than the southern part. In Ethiopia and Ivory Coast, the prevalence was 55% and 39%, respectively. The prevalence of MRSA was lower than 50% in most of the African countries, although it appears to have risen since 2000 in many African countries, except for South Africa (30).

Scott K.H et al in S.Africa have conducted a study on Prevalence of methicillin-resistant *S.aureus* nasal carriage among hospitalized patients with tuberculosis and they found that out of 52 patients with an admission nasal swab, 11 (21%) were positive for MRSA. An additional 4 (10%) of patients with negative admission swabs were positive for MRSA on repeat testing. MRSA carriage on admission was more common among patients with previous hospitalization, and among HIV-infected patients was significantly associated with lower CD4 counts (p=0.03). All MRSA isolates were resistant to cotrimoxazole, and 74% were resistant to ≥5 classes of antibiotics; all retained susceptibility to vancomycin (31).

Another research done in Kenya by Aiken e.t al. on the carriage of *S.aureus* in Thika Level 5 Hospital. In this typical mid-sized Kenyan Government hospital, they performed 950 screens for current carriage of *S.aureus* amongst inpatients over a four month period. They detected *S.aureus* carriage (either MSSA or MRSA) in 8.9% (85/950; 95%CI 7.1-10.8) of inpatient screens, but patients with multiple screens were more likely have detection of carriage. MRSA carriage was

rare amongst *S.aureus* strains carried by hospital inpatients – only 7.0% (6/86; 95%CI 1.5-12.5%) of all isolates were MRSA (32).

With regard to Ethiopia, there are few studies done on MRSA among different study subjects.

There was meta-analysis study on MRSA in Ethiopia by Eshetie et al during 2004-2015 and by this study the pooled prevalence of methicillin resistant *S.aureus* was 32.5%. Moreover, MRSA strains were found to be highly resistant to penicillin, ampicillin, erythromycin, and amoxicillin, with a pooled resistance ratio of 99.1, 98.1, 97.2 and 97.1%, respectively. On the other hand, comparably low levels of resistance ratio were noted to vancomycin, 5.3% (33).

Tibebu et al have conducted a study on MRSA among HIV Infected Pediatric Patients in Northwest Ethiopia: And by this study *S.aureus* was detected in 206 participants (51.5%). The prevalence of MRSA colonization in this study was 16.8%. Colonization by *S.aureus* was associated with male gender, history of antibiotic use over the previous 3 months and having CD4 T cell counts of more than 350 x 106 cells / L. Concomitant resistance of the MRSA to clindamycin, chloramphenicol, co-trimoxazole, ceftriaxone, erythromycin and tetracycline was 7.6%, 6%, 5.25%, 20.9%, 23.9% and 72.1%, respectively (34).

The latest study done by Gebremedhn et al. on prevalence and risk factors of MRSA colonization among HIV patients in Mekelle from September 2014 to February 2015. By this study out of 249 study participants, *S.aureus* was isolated from 81 (32.5 %) patients, with MRSA colonization rate of 6 (2.4 %). MRSA isolates were resistant to Ciprofloxacin and trimethoprim-sulphamethoxazole (16.7 % each), clindamycin (33.3 %) and erythromycin (50 %). However, all MRSA isolates were 100 % sensitive to Amikacin. History of hospitalization, percutaneous device usage, patients with a household member's hospitalization and low CD4 count (<200 cells/mm3) were significantly associated with *S.aureus* colonization ($p < 0.05$) (35).

3. Objectives

3.1 General objective

To determine the prevalence and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* among Clinically TB suspected clients at St. Peter Hospital Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the prevalence of methicillin-resistant *Staphylococcus aureus* on Clinically TB suspected clients.
- To identify factors associated with colonization of methicillin-resistant *Staphylococcus aureus*.
- To determine the antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates.

3.3 Hypothesis

There is no difference between the findings of this study and previous studies about prevalence of MRSA on clinically TB suspected clients.

4. **Materials and Methods**

4.1 Study area

The study was conducted at St. Peter Hospital which is found in Addis Ababa, the capital city of Ethiopia. The town is located in the foothills of the Entoto Mountains and standing 7,726 feet (2,355 meters) above sea level, which is the third highest capital in the world with a population of 3,627,934. St. Peter's TB Specialized hospital is one of the governmental hospitals operating under the Ministry of Health in Ethiopia. It is one of the main tuberculosis hospitals in Africa. In addition to tuberculosis diagnosis and management, the hospital is providing different services including Voluntary counseling and testing, health-facility based medical care, Psycho-Social counseling and support, information, education and communication and HIV/AIDS research. It has a total of 277 beds of which 68 are exclusively designated to TB patients.

4.2 Study Design and period

A prospective hospital-based cross sectional study was conducted from April to July, 2018.

4.3 Population

4.3.1 Source Population

All clients who visited St Peter Hospital during the study period.

4.3.2 Study Population

Consecutive adults presented to the study area during the study period with suspected TB that fulfilled the inclusion criteria were included.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Patients with age of ≥18 years.
- Patients with signs and symptoms suggestive of pulmonary TB.
- Provision of informed consent to participate in the study.

4.4.2 Exclusion criteria

- Participants on anti- tuberculosis treatment
- Any of our clients who are critically ill and not volunteers was excluded.
- Clients with clear nasal infection.
- Participants who were on antibiotic treatment for any bacterial infection during the time of data collection were excluded from the study.
- Study participants who had nasal bleeding at the time of data collection were excluded because rolling of swab may aggravate bleeding.

4.5 Study Variables

4.5.1 Dependent Variables

- Prevalence of MRSA
- Antimicrobial susceptibility pattern

4.5.2 Independent Variables

- age
- sex
- education level
- occupation
- And associated factors

4.6. Sample size and sampling technique

4.6.1. Sample size calculation

The sample size was calculated using single proportion formula by taking the prevalence of MRSA from South Africa, which was 21 % (31):

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

n = sample size

Z α/2= 95% CI two tailed (1.96)

d= desired precisions = 5% , P= 21% prevalence of MRSA in TB patients (31).

Thus, the study was included at least 255 subjects, but assuming 10 % non-response rate, the sample size was: n=255+10 %= 281.

4.6.2 Sampling technique

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

4.7 Measurement and Data collection

4.7.1 Data collection procedure

Data collectors was identified, oriented and informed to collect the data as per the pre-structured questionnaire. The purpose of the study as well as any related harm and benefit was explained to the study participants accordingly.

To identify the potential associated factors for MRSA colonization, clinical information was collected from each participant. For each participant, with or without the assistance of their health care workers and from their medical records (In addition, interviewers extracted relevant data from their patient medical records), we collected the demographic data, personal history, dialysis status, chronic wounds, and insertion of medical devices and antibiotic use, hospitalization and surgery in the previous 3 months was also be collected.

4.7.2 Laboratory Methods

4.7.2.1 Nasal swabs collection, transportation *and* storage

Nasal swabs were collected from the St. Peter Hospital as per the collection and transportation policy of the Ethiopian Public Health Institute (EPHI) National Bacteriology Laboratory. The swabs were collected using sterilized cotton swab and introduced the sterilized cotton swab 2-3 cm in the nasal cavity and rotated 4-5 times both clockwise and anticlockwise before withdrawal. A specimen was obtained from each participant from both anterior nares consecutively using the same swab and placed into amies transport media and transport by triple packaging system to EPHI National Bacteriology Laboratory. Each specimen was stored at refrigerator until the end of this research project (36).

4.7.2.2 S.aureus isolation and Identification

All swabs were inoculated on both Sheep Blood Agar (SBA) and mannitol salt agar (MSA) and incubated at 35ºC for 24-48 hours. *S.aureus* was identified based on the colony morphology on MSA and SBA, gram staining, catalase test and tube coagulase test. (37).

4.7.2.3 MRSA detection

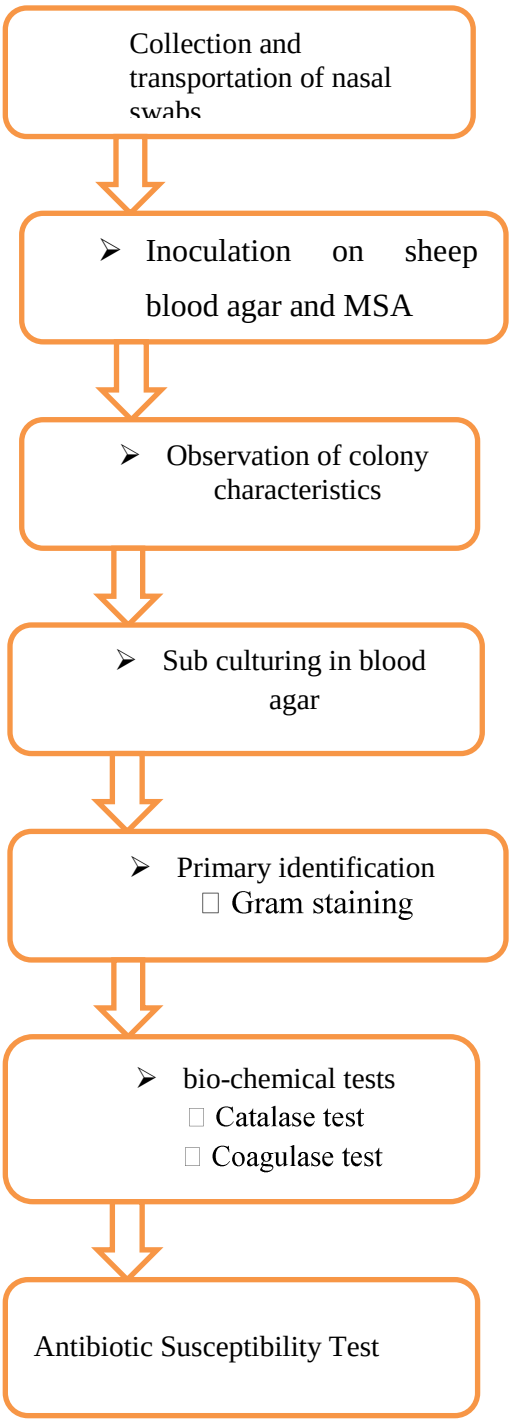
Methicillin sensitivity/resistance of each S.aureus was tested using surrogate antibiotics of 30µg cefoxitin discs by the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI). Inocula were adjusted at 0.5% McFarland standard and each streaked uniformly with swab sticks in Mueller-Hinton agar plates. The plates were allowed to dry for 3-5 minutes before the disks used. The plates were then incubated aerobically at 35°C for 24 hrs. The zone of inhibition was interpreted according to CLSI 2018 guidelines. All isolates resistant to cefoxitin were considered as MRSA. An inhibition zone of 21 mm or less around cefoxitin disc indicated MRSA. S. aureus ATCC 25923 was used for quality control. (36).

4.7.2.4 Antimicrobial susceptibility testing (AST)

The antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion technique. Select 3 – 5 well-isolated colonies of the same morphologic type from an agar plate culture and transfer the growth into a tube containing 4 – 5 ml of normal saline solution (NSS) to make direct colony suspension of the isolates. Mix well and adjust turbidity with NSS to match 0.5 McFarland standards. A standardized inoculum of the bacteria was swabbed onto the surface of a Mueller Hinton agar (MHA) plate. Filter paper disks impregnated with antimicrobial agents were placed on the agar. After overnight (24hr) incubation at 35+ 0r -2°C, the diameter of the zone of inhibition around each disk was measured. By referring to the standardized tables compiled by CLSI 2018 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 Units), Cefoxitin (30µg), Erythromycin (15µg), Clindamycin (2µg), Trimethoprim –Sulphamethoxazol (1.25/23.75µg), Tetracycline (30µg), Ciprofloxacillin (5µg), Chloramphenicol (30µg), Gentamicin (10µg), Vancomycin-MIC. *S.aureus* (ATCC-25923) was used as control for the antimicrobial susceptibility pattern.

In addition, inducible clindamycin resistance was also tested by disk diffusion using D-zone test as per CLSI guidelines. Briefly, erythromycin (15µg) disk was placed at a distance of 15–26 mm (edge to edge) from clindamycin (2µg) disk on a MHA. After overnight incubation, plates were examined for the formation of flattened zone of inhibition adjacent to the erythromycin disk. Formation of D-shape with erythromycin indicated a positive clindamycin inducible resistant (34).This test method is applied only to organisms which are resistance to Erythromycin and susceptible or intermediate to Clindamycin (38).

Fig.4.1 Flow chart for isolation and identification of S.aureus from Nasal swabs



4.8 Data Quality Assurance

4.8.1 Pre analytical

Data collection was conducted after the data collectors were given orientation and participants being informed about the purpose of the study and when given consent. Standard operating procedures (SOPs) of the host laboratory had been ensured the reliability and validity of test result. The nasal swab collection containers were labeled and dated. The specimens were collected, transported as quickly as possible and in a container, such as triple packaging system and stored according to the SOP. All patient information collected during the study period was checked for its clarity and completeness in a regular basis. Each lot of the medium was checked for expiration dates prior to use as part of quality control.

4.8.2 Analytical

All laboratory tests were performed by well-trained laboratory personnel. Standard operational procedures of the host laboratory had been ensured the reliability and validity of test result. All Samples brought to the testing bench were analyzed as soon as possible. The performance of the media and antibiotic discs were evaluated using positive controls standard American Type Culture Collection (ATCC) reference strain of *S.aureus* (*S.aureus*, ATCC-25923).

4.8.3 Post analytical

SOPs of the host laboratory were used to ensure the reliability and validity of test result. All laboratory results were recorded on a Laboratory Analysis Result Form during the study period. The left over Samples were stored at -2-8°c.And the isolates are stored at -80°c for any kind of checkup and future use.

4.9 Data analysis and interpretation

Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) software version 23. Descriptive statistics, frequency and percentage, were used to describe the study participants in relation to relevant variables. Univarate and multivariate logistic analysis were performed to assess the association of the risk factors associated with *S.aureus*/MRSA colonization. In all cases P-value less than 0.05 were considered as statistically significant. Finally, the results were presented on words, figures and tables.

4.10 Ethical considerations

The study was approved by Research and Ethics Review Committee of the Department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences; Addis Ababa University. From the department, a letter asking cooperation to St. Peter Hospital and EPHI National Bacteriology Laboratory was written. Accordingly, permission was obtained from St. Peter Hospital and EPHI National Bacteriology Laboratory to collect data from study population and for Laboratory analysis respectively. All eligible subjects who gave written informed consent were enrolled in the study. All the information obtained from the study subjects were coded to maintain confidentiality.

4.11 Dissemination of the result

The finding of this study was disseminated to the department of Medical Laboratory Sciences, School of Allied Health Sciences, College of Health Sciences; Addis Ababa University and St. Peter Hospital. In addition the research will be presented to other concerned bodies like professional associations and it will be submitted to peer reviewed journals for publication.

4.12 Definition of Terms

Antibiotic- is a substance produced by a microorganism that has the capacity, to selectively inhibit or kill other microorganisms (Paul Vuillemin, 1941)

Antimicrobial Resistance (AMR) - is the ability of microbes to grow in the presence of a chemical (drug) that would normally kill them or limit their growth.

Clinically TB Suspected - Any person who presents with symptoms or signs suggestive of TB.

CA-MRSA- MRSA isolates were considered to be community acquired if they were recovered within 72 h of admission to the Hospital.

Colonization- In the absence of any potential source or clinical evidence of infection, MRSA was considered to be colonizing the site from which a specimen was obtained.

HA-MRSA- MRSA isolates were considered to be health care- acquired if they were recovered after 72 h of admission to the Hospital.

MDR- resistant to three or more antimicrobial class in addition to Cefoxitin/ Oxacillin.

Susceptibility - the quality or state of being susceptible; the state of being predisposed to, sensitive to, or of lacking the ability to resist something.

5. Result

5.1 Socio demographic characteristics

Among 281 study participants included, 164 (58.4%) were females. The study participant's age was ranged from 18 to 93 years old with the mean standard deviation of 36.8±16.Most of the study participants 94 (33.5%) were at primary educational status, others 83 (29.5%),62 (22.1%),42 (14.9%) were secondary, illiterate, higher educational status respectively. And once again majority of the participants 147 (52.3%) had no work (Table 5.1).

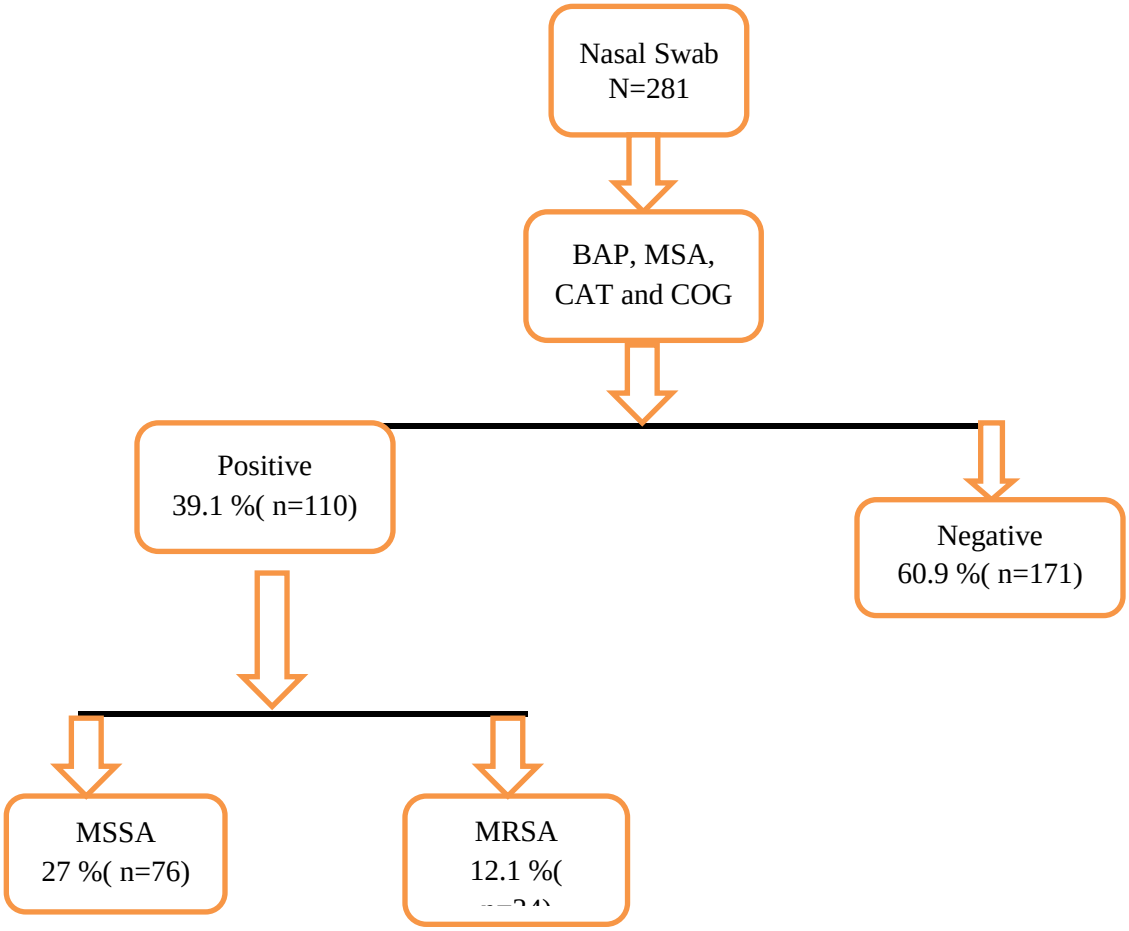
Table 5.1 Socio demographic characteristics of clinically TB suspected clients at St. Peter Hospital from April to July 2018 (n=281).

Variables	Category	Frequency (%)
Gender	Female	164 (58.4%)
	Male	117 (41.6%)
Age	18-28	110 (39.1%)
	29-39	72 (25.6%)
	40-49	45 (16.0%)
	>49	54 (19.2%)
Educational level	Illiterates	62 (22.1%)
	Primary	94 (33.5%)
	Secondary	83 (29.5%)
	Higher	42 (14.9%)
Occupation	Governmental	36 (12.8)
	Private	75 (26.7%)
	Merchant	23 (8.2%)
	No	147 (52.3%)

5.2 Prevalence of nasal *S.aureus* / MRSA colonization.

From the total 281 nasal cultures, *S.aureus* was recovered from nasal swabs taken from 39.1% (n=110) participants, of whom 12.1% (n=34) were MRSA while 27.0% (n=76) were MSSA. *S.aureus* was not detected in 60.9% (n=171) study participants. MRSA accounted for 30.9% (n=34) of 110 *S.aureus* isolates.

Fig.5.1. Prevalence results Summary.



(Key; n=number of participants, BAP=Blood agar plat, MSA=manitol salt agar, CAT=Catalase and COG=Coagulase)

Of the total 34 (30.9%) MRSA colonization, male accounts 12 (10.9%) and female accounts 22 (20%). Like MRSA colonization, there was high colonization rate of *S.aureus* among female 69(24.6%)) and the rest 41(14.6%) were male .MRSA colonization rate was almost equally distributed in each age group (table 5.2).

Table 5.2: Age and sex distribution of *S.aureus* and MRSA among clinically TB suspected clients at St. Peter Hospital from April to July 2018 (n=281).

Study characteristics		***S.aureus* colonization**		**MRSA colonization**	
		Yes	**No**	**Yes**	**No**
		No. (%)	**No. (%)**	**No. (%)**	**No. (%)**
Gender	**Female**	69(24.6%)	95(33.8 %)	22 (20%)	47 (42.7%)
	Male	41(14.6%)	76(27.0%)	12 (10.9%)	29 (26.4%)
Age (year)	**18-28**	47 (16.7%)	63 (22.4%)	10 (9.1%)	37 (33.6%)
	29-39	25 (8.9%)	47 (16.7%)	8 (7.3%)	17 (15.4%)
	40-49	17 (6.0%)	28 (10.0%)	7 (6.4%)	10 (9.1%)
	>49	21 (7.5%)	33 (11.7%)	9 (8.2%)	12 (10.9%)

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

Variables assessed as risk factors and found with a frequency of less than 10 % of the total 281 participants were excluded from the statistical logistic analysis of risk factors for *S.aureus*/MRSA. These include history of surgery in the past 1 year (9.6%) and Dialysis status in the past 1 year (3.6%).

Individuals in the age group of 18-28 years were more colonized by *S.aureus*; however, there was no statistical significance (Table 5.3). *S.aureus* colonization rate was higher among females 69 (24.6 %) as compared to males 41 (14.6 %). However, this was not statistically significant (p = 0.234) (Table 5.3). Among 34 MRSA isolates, 22 (20 %) were from females and 12 (10.9%) were from male (Table 5.4). However, this was not statistically significant (p = 0. 774) (Table 5.4). By bivariate Analysis, *S.aureus* colonization rate was statistically significant in individuals with the history of hospitalization (OR = 2.386; 95% CI: 1.452–3.887; p-value = 0.001) and percutaneous device usage within the past 1 year (OR = 2.285; 95% CI: 1.289–4.050; p-value = 0.005) (Table 5.3). Risk factors for the colonization of *S.aureus* with statistical significance in the bivariate analysis were further analyzed using multivariate analysis and were found to be significantly associated for hospitalization (OR = 2.222; 95% CI: 1.209–4.085; p-value = 0.010) but not for percutaneous device usage within the past 1 year (OR = 1.447; 95% CI: 0.720–2.910; p-value = 0.300) (Table 5.5). In bivariate logistic regression analysis (Tables 5.4), there were no significant associations between MRSA colonization and the independent variables.

Table 5.3 bivariate Analysis of risk factors for Nasal colonization of *S.aureus* in clinically TB Suspected individuals attending St. Peter Hospital in Addis Ababa, Ethiopia, April – July 2018.

variable	category	Frequency No (%)	*S.aureus* colonization		
			No (%)	OR (95%CI)	P value
Age in years	18-28	110 (39.1%)	47 (42.7%)	0.85(0.439-1.658)	0.64
	29-39	72 (25.6%)	25 (22.7%)	1.196(0.576-2.486)	0.63
	40-49	45 (16.0%)	17 (15.5%)	1.048(0.464-2.365)	0.91
	>49®	54 (19.2%)	21 (19.1%)		
Gender	Female®	164 (58.4%)	69 (42.1%)		
	Male	117 (41.6%)	41 (35.1%)	1.346(0.825-2.198)	0.234
Hospitalization	No®	166 (59.1%)	51 (30.7%)		
	Yes	115 (40.9%)	59 (51.3%)	2.386(1.452-3.887)	.001
Household member's hospitalization	No®	215 (76.5)	79 (36.7%)		
	Yes	66 (23.5%)	31 (47%)	1.525(.873-2.662)	0.138
Presence of indwelling medical device	No®	219 (77.9%)	76 (34.7%)		
	Yes	62 (22.1%)	34 (54.8%)	2.285(1.289-4.050)	0.005
Oral antibiotic usage	No®	65 (23.1%)	21 (31.8%)		
	Yes	216 (76.9%)	89 (41.2%)	1.468 (.817-2.639)	0.199
HIV Status	Negative®	223 (79.4%)	89 (39.9%)		
	Positive	58 (20.6%)	21 (36.2%)	0.855 (0.470-1.555)	0.607
Chronic Wound	No®	246 (87.5%)	104 (42.3%)		
	Yes	35 (12.5%)	6 (17.1%)	0.282(0.113-0.705)	0.007

OR=Odds Ratio, ® = Reference category and No. =Number of *S. aureus* in each category

Table 5.4 Bivariate Analysis of risk factors for nasal colonization of MRSA in clinically TB Suspected individuals attending St. Peter Hospital in Addis Ababa, Ethiopia, April –July 2018.

Variables	category	Frequency No. (%)	MRSA colonization		
			No.	OR(95%CI)	P-value
Age in years	18-28	110 (39.1%)	10	0.36(0.119-1.095)	0.072
	29-39	72 (25.6%)	8	0.627(0.188-2.095)	0.449
	40-49	45 (16.0%)	7	0.930(0.255-3.411)	0.917
	>49®	54 (19.2%)	9		
Gender	Female®	164 (58.4%)	22		
	Male	117 (41.6%)	12	0.884(0.38-2.05)	0.774
Hospitalization	No®	166 (59.1%)	20		
	Yes	115 (40.9%)	14	0.48 (0.2-1.097)	0.082
Household member's hospitalization	No®	215 (76.5)	21		
	Yes	66 (23.5%)	13	0.501 (0.210-1.197)	0.120
Presence of indwelling medical device	No®	219 (77.9%)	26		
	Yes	62 (22.1%)	8	1.690 (0.671-4.255)	0.265
Oral antibiotic usage	No®	65 (23.1%)	5		
	Yes	216 (76.9%)	29	0647 (0.216-1.938)	0.436
HIV Status	Negative®	223 (79.4%)	28		
	Positive	58 (20.6%)	6	1.148 (0.403-3.270)	0.797
Chronic Wound	No®	246 (87.5%)	34		
	Yes	35 (12.5%)	0		0.999

OR=Odds Ratio, ® = Reference category and No. =Number of MRSA in each category

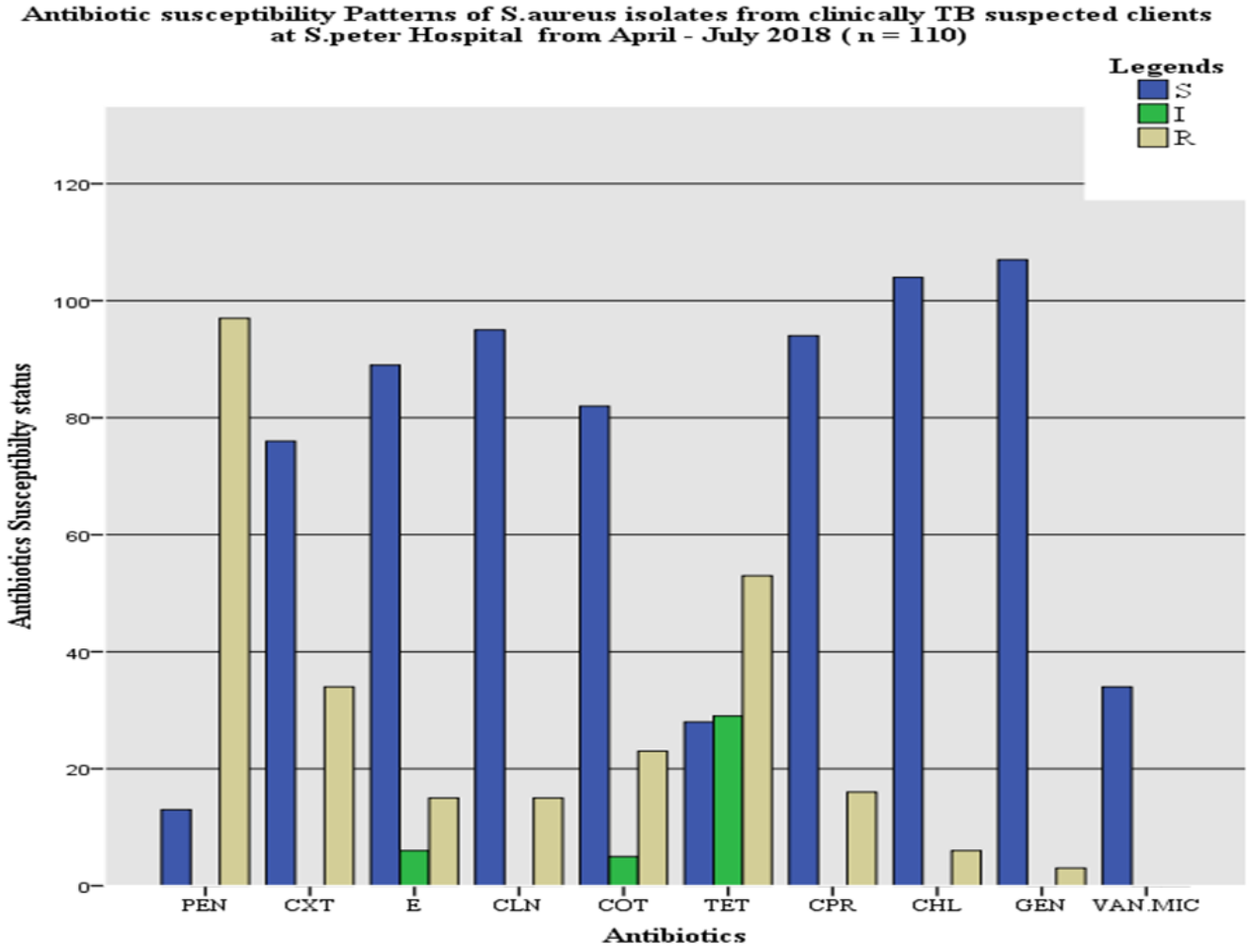
Table 5.5 Multivariate analysis of independent risk factors associated with *S. aureus* colonization in clinically TB Suspected individuals attending St. Peter Hospital in Addis Ababa, Ethiopia, April –July 2018.

Risk factors	AOR (95 % CI)	P value
Hospitalization	2.222 (1.209-4.085)	0.010
Presence of indwelling medical device	1.447 (0.720-2.910)	0.300

5.4. Antibiotic Susceptibility Tests for *S.aureus* and MRSA

All the isolated of *S.aureus* were tested for susceptibility to selected antibiotics. Antimicrobials used in this study were, Penicillin, Cefoxitin, Erythromycin, Clindamycin, Trimethoprim - Sulphamethoxazol, Tetracycline, Ciprofloxacillin, Chloramphenicol, Gentamicin, Vancomycin - MIC. The present study has demonstrated the existence of the levels of resistance of *S.aureus* to commonly used antimicrobial agents in the study area. Significant proportions of *S.aureus* isolates (94.5%) were susceptible to Chloramphenicol, Penicillin (11.8%), Clindamycin (86.4%), Erythromycin (80.9%), and Vancomicin- MIC (100%) as shown in (Fig.5.2).

Fig. 5.2: Antibiotic-resistance profiles of *S.aureus* isolates (N=110) from nasal swabs.



30.9% (n=34) of the *S.aureus* was found to be resistance to Cefoxtin, which shows the prevalence of MRSA. All 34(100%) MRSA isolates were resistant to penicillin and sensitive to vancomycin-MIC. The susceptible profile of Erythromycin, Clindamycin(CLN), Gentamicin, Tetracycline and Vancomycin of MRSA isolates were 64.7%, 67.6%, 91.2%,,11.8% and 100% respectively (Table 5.6).From the total of all MRSA (n=34), 11 were CLN resistance and two of CLN resistance were detected using the D-zone test. So the prevalence of inducible CLN resistance was 5.9% (n=34).

Table 5.6: Antibiotic susceptibility pattern of MRSA Isolates (N=34) among clinically TB suspected clients at St. Peter Hospital, Addis Ababa, Ethiopia.

Antibiotic	S	I	R	% of R
Cefoxitin	0	0	34	100
Penicillin	0	0	34	100
Trimethoprim-Sulphamethoxazol	23	0	11	32.3
Tetracycline	4	5	25	73.5
Gentamicin	31	0	33	8.8
Ciprofloxacin	26	0	8	23.5
Erythromycin	22	1	11	32.3
Clindamycin	23	0	11	32.3
Chloramphenicol	29	0	5	14.7
Vancomycin- MIC	34	0	0	0

S = susceptible; I = intermediate; R = resistant.

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5.5 Drug resistance pattern/ antibiogram of multi drug resistance MRSA

The resistance patterns of multi drug resistance (MDR) MRSA isolates varied from three to six drugs and 17 of the MRSA isolates (50 %) have shown multidrug resistance. The highest resistance pattern (17.6%) was observed for three antibiotics with pattern of E/CLN/TET. Three resistance pattern (COT/TET/CHL/GEN) was observed to for four antibiotics. Similarly, three resistance pattern (E/CLN/COT/TET/CPR) was observed to five antibiotics. Maximum resistance (resistant to six drugs) was observed for two MRSA isolate with resistance pattern of E/CLN/COT/TET/CPR/CHL.

Table 5.7: Drug resistance pattern of MRSA isolates for different antibiotics among clinically TB suspected clients at St. Peter Hospital, Addis Ababa, Ethiopia.

No of antibiotics	Drug resistance pattern	Resistant strains (No (%))
3	COT/TET/CPR	3 (8.8%)
	E/CLN/TET	6 (17.6%)
4	COT/TET/CHL/GEN	3 (8.8%)
5	E/CLN/COT/TET/CPR	3 (8.8%)
6	E/CLN/COT/TET/CPR/CHL	2 (5.9%)

E=Erythromycin, CLN=Clindamycin, COT=Trimethoprim -Sulphamethoxazol, TET=Tetracycline, CPR=Ciprofloxacillin, CHL=Chloramphenicol, GEN=Gentamicin

6. Discussion

This study is the first of its kind to assess rates of MRSA colonization of clinically TB suspected clients in the study area particularly and in Ethiopia at large. The findings underscore the existence of alarming rates of MRSA colonizers that are simultaneously resistant to some commonly prescribed antimicrobial agents.

In the present study, the overall prevalence of isolation of *S.aureus* from clinically TB suspected clients was 39%. The finding of this study was in agreement with, a related study done in Tanzania (39), reported an isolation rate of 34.5% from nasal swabs of admitted patients and a study conducted in Iran, (40), the rate of isolation of *S.aureus* from Patients Admitted to the Infectious Diseases Ward of Imam Hospital was 39.8%.

The findings of *S.aureus* isolations (39%) rate in our study was higher than that of the study conducted in S. Africa (31), Georgia (29) and Kenya (32) where the isolation rate was 25%, 23.7% and 8.9% respectively. The high prevalence of *S.aureus* colonization in our study might be due the more visit of these clinically TB suspected clients to the health facilities (since repeated visits to health care centers, or contact with hands of health care workers in clinically TB suspected individuals is the major risk factor for the colonization), transportation issue (we transport the nasal swabs by triple packaging system using ice box but the first study transport simply at room temperature), in isolation techniques (the last two studies inoculated the swabs to MSA and sub-culture to blood agar), and difference in personal hygiene, geographical distribution and population characteristics.

Our rate of S.aureus isolation (39%) is lower than that of the study conducted in Northwest Ethiopia (34) where the isolation rate was 51%. The low prevalence of *S.aureus* colonization in our study might be due to difference in sample collection techniques (they collect 3 types of samples for each participant and use sterile broth moistened swabs), geographical area and study population.

In our study, the prevalence of MRSA isolation (12%) was lower than that of the study conducted in S.Africa (31), and Iran (40) where the isolation rate was 21% and 59% respectively. This increased isolation rate of MRSA could be due to differences in isolation technique

(multiple screens) and there might be specific risk factors which are not assessed by this study that might increase the isolation rate.

Our MRSA prevalence of 12 % was higher than to the 8.5%, 7.3%, and 0.63%% reported from Tanzania (39), Georgia (29), and Kenya (32). This increased isolation rate of MRSA in our research project could be due to differences in isolation technique (the second study used Oxacillin disk to detect MRSA which is affected by test conditions) and there might be specific risk factors which are not assessed by this study that might increase the isolation rate.

In this study, recent hospitalization was associated with *S.aureus* colonization. This was also showed by other studies conducted in Georgia (29), Mekelle; Ethiopia (35) and Iran (40). Presence of percutaneous device within the past year was found to have association by bivariate analysis with *S.aureus* colonization. This result was supported by other studies conducted in Mekelle, Northern Ethiopia (35).

In the current study, Colonization by MRSA was not associated with any one of the variables. And this finding was consistent with the study done in Northwest Ethiopia (34) where Colonization by MRSA was not associated with any one of the variables. In general, there is no common consensus for risk factors of MRSA among different geographical area around the globe.

In our study the antimicrobial susceptibility pattern of MRSA isolates showed 100 % sensitivity to Vancomicine-MIC and 100% resistance to Penicillin which was similar to the study in S.Africa among hospitalized patients with tuberculosis (31).

Significant proportions of MRSA isolates (85.6%) were susceptible to Chloramphenicol, Ciprofloxacin (76.5%), Clindamycin (67.6%), Erythromycin (64.7%), Gentamycin (91.2%), Trimethoprim-sulphamethoxazole (67.6%) and Tetracycline (11.8%). Comparable to our result, a study conducted in S.Africa reported that, 17% of MRSA isolates were sensitive to Tetracycline (31)**.**But incomparable to our result, a study conducted in S.Africa (31) showed that, 0%,0%, 26% and100% of MRSA isolates were sensitive to Gentamycin, Trimethoprim-sulphamethoxazole, Erythromycin and Clindamycin respectively. The prevalence of inducible CLN resistance by this study was 5.9% (n=34).Unlikely to this result, the study conducted in

Dares Salaam, Tanzania (39) reported the prevalence of inducible CLN resistance as 31.8% (n=22).

In our study 17 of the MRSA isolates (50 %) have shown multidrug resistance which was similar to the study conducted in Mekelle; Ethiopia (35) which reported the prevalence of MDR-MRSA as 50%, however, this findings was lower than that of the study conducted in Dares Salaam, Tanzania (39) reported the prevalence of MDR-MRSA as 72.7%.

Differences in susceptibility pattern of the isolates could be due to differences in geographical area and the time where MRSA isolates first occurred (the longer the MRSA are present in a geographic area, the more likely they are to be resistant to other antibiotic classes). MRSA isolates were highly susceptible to most tested antibiotics, these could be due to CA-MRSA strains were capable of resisting only β- lactam antibiotics as the result of carriage of genetic element SCC*mec* type IV. *SCCmec* type IV is one of the shorter SCC*mec* variations less likely to carry multi-drug resistance (41). A few MRSA isolates showed resistance to the tested drug, these might be due to the possible mixing of HA-MRSA and CA-MRSA (42).

7. **Strength and limitation of the study**

7.1 Strength

- To the best of our knowledge, this is the first attempt to determine the prevalence of MRSA on the clinically TB clients in the study area and in Ethiopia.
- We used VAN.MIC test for MRSA Isolates.
- It was done on National Bacteriology Laboratory which is well equipped and had an access of quality control strains and materials for each step of laboratory part.

7.2 Limitation.

- Our data was collected at a public urban hospital with a high prevalence of TB, HIV and TB-HIV infection. Thus, generalization of these findings to other populations should be done with caution.
- This study limited to conduct the MRSA gen as well as some additional antimicrobial agents.

8. Conclusion

In this study there was a higher prevalence of *S.aureus* and MRSA colonization among the study subjects. History of hospitalization was significantly associated with S.aureus colonization. In the current study, Colonization by MRSA was not associated with any one of the variables. In this study MRSA strain was found to be highly resistant to penicillin. In contrary, low level of resistance rate was observed to vancomycin. Thus, to combat the burden of MRSA in particular, the following concerns should be considered at the national level, such as adopting safety protocols and implementing proper antibiotic prescription policies.

9. **Recommendations**

- Different epidemiological risk factors that interplay in colonization of S.aureus/MRSA should be studied routinely which ultimately downgrading the prevalence of the disease.

- Risk factor controlling mechanisms should be implemented.

- Medical researchers should make further investigation on public health significance of MRSA at regional and national level.

- In principle, appropriate use of antibiotics, applying safety precautions are the key to reduce the spread of multidrug resistant strains, MRSA in particular.
- Medical practitioners should be encouraged to choose antibiotic based on susceptibility test and to wear protective equipment during screening and handling of patients to reduce contamination and spread of the disease.

- The Hospital should develop guidelines for the prevention and control of MRSA

- Molecular analysis of the methicillin resistant S.aureus bacterial isolates for strain typing is planned as a continuation of this study

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11. Annexes

Annex I: English Versions of Participant Information Sheet

My name is Akele. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled **Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-Resistant Staphylococcus aureus (MRSA) colonization among Clinically TB suspected clients.** You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

Introduction

The topic of this study is Prevalence, and Antimicrobial Susceptibility patter of Methicillin-Resistant Staphylococcus aureus (MRSA) colonization among clinically TB suspected clients. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences. If you decide to participate, you have to sign on the consent form and you may obtain a copy of this information sheet.

Expected from participants

As a participant of this study, you are expected to give Nasal Swab. Being asked to give sample does not neccessarly mean that you have the disease. When you are found to be positive for the micro-organism, you will be informed by the health worker and receive proper treatment.But 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.

Confidentiality

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.

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 the study is useful for better understanding of the problems of **clinically TB suspected clients**. You will also obtain all the results of the analysis and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are: **Investigator: Akele Kubi** (BSc, MSc student), St.Peter Hospital: +251913835564

Email- akelekubiteferi@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences: Telephone +251112755170

Annex II: Informed consent form (English version)

Dear participant, if you are agreed to take part in this study based on the information given to you, please listen or read this form one by one and tick every box to show your agreement on each points and sign the consent sheets at the end of this form. If there is any unclear point, don't hesitate to ask question until you understand it to make your decision by your own interest.

1. I know the objective and procedure of this study after I have read, or it was read to me, the information sheet concerning this study and I understand what will be required of me if I take part in the study.	□
2. I understand that being participation on this study is voluntary; confidentiality of my personal information is guaranteed.	□
3. I understand that at any time I have the right to withdraw from this study without giving a reason.	□
4. I know the information regarding TB disease after he/she explain for me the procedure of collection,	□
5. The interviewer explain for me as there is no any risk or discomfort, and extra treatment	□
6. 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.	□
7. I know there is no extravagant to me without time taken for interview, counseling and health education.	□
8. I know that, if there are any physical, mental problems due to participating in the study, all responsibilities are come to project manager	□
9. I know that, if there is any disagreement with the procedure of the study I will appear to research ethical clearance board of the Ethics Committee of the Departmental Research and Ethics Review Committee of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory Sciences.	□

I understand all the information given above and I agreed to participate in this study by my full Interest. And I assure my agreement by my official signature.

Signature: ----------, Date: ----------/--------/----------

Participant Phone Address if possible: ----------

I project manager ,Akele Kubi, agreed on all commitment on this form to fulfill all safety procedure, right and benefit for the participants, and then I assure my agreement by my official signature

Project Manager Name: Akele Kubi

Phone Number: +251913835564; Work address: St. Peter hospital, Addis Ababa, Ethiopia.

Signature: ----------, Date: -------/------/---------

Annex III: Informed consent form (Amharic version)

በጥናቱ ለመሳተፍ ፍቃደኛ ከሆናችሁ ፎርሙን በሚገባ ከነበባችሁ/ከተነበበላችሁ በ□ላ በ□ርሙ ላ□ በሚቶ□ ክፍት ሳጥኖች ላይ ምልክት በማድረግና በፎርሙ መጨረሻ ላይ በሚገኘው ክፍት ቦታ ላ□ በመ□ረም እንድተረጋግጡልን እንጠይቃለን። ያልተረዳችሁት/ያልገባችሁ ነገር ከለ እባኮት ከመጠየቅ ወደ □ላ አይበሉ።

1. **የጥናቱ ዋና አላማና አተገባበሩ ከነበብኩኝ /ከተነበበልኝ በ□ላ በጥናቱ ብሳተፍ ከኔ ምን □እንደሚጠበቅ ተረድቻለሁ**	□
2. **በጥናቱ የምሳተፈው በፍቃዴ ነው።የምሰጠው መረጃ በጥብቅ እንደሚቀመጥ ተረድቻለሁ።**	□
3. **በጥናቱ መቀጠል ከልፈለኩ በማንኛ□ም ቂ□ ማቋረ□ እንደምችል ተረድቻለሁ።**	□
4. **የሳንባ ነቀርሳ ምንነትና ለጥናቱ የሚሆን ናሙና እንዴት አንደምሰጥ ገለጻ ተደርጎልኛል።**	□
5. **በጥናቱ በመሳተፌ የሚደረስብኝ ጉዳት እንደሌለ ጠያቂ□ አስረድቶኛል።**	□
6..**ያለኔ ፍቃድ የጥናቱ ውጤት የግል መረጃዎቼን ይፋ እንደማያደርግ ተረድቻለሁ።**	□
7..**ምንም አየነት የገንዘብ ወጪ እንደሌለብኝ ከጠያቂየ ተረድቻለሁ**	□
8. **በአሰራሩ ላይ ችግር ባይበት/ባገኝበት ቅ□ስ ቻ□ሮስ ሆስፒል ኢቲከል ኮሚ ማሳወቅ እንደምችል ተረድቻለሁ።□**	□

ከላይ የተዘረ□ሩት መረጃዎች ተረድቼ በጥናቱ ለመሳተፍ የተስማማሁ መሆኑን በፊርማ□ አረጋግጣለሁ።

ኝርማ: ----------, **ቀን**: ----------/--------/2017

ስል□ ቁ□ር/ ከለ/: ----------

የጥናቱ ተሳታፊዎች ደህንነት፤መብትና ጥቅም የተጠበቀ መሆኑን በፊርማየ አረጋግጣለሁ።

ኝርማ……..

የጥናቱ ባለቤት፦

ስል□ ቁ□ር:-251913835564---------- **የስራ አድራሻ**: **ቅዱስ ጴጥሮስ ሆስፒተል አዲስ አበባ ኢትየጵያ**

ኝርማ: ----------, **ቀን**: -------/------/

Annex IV: Questionnaires

Questionnaires to collect demographic data and associated factors of MRSA of participants.

I. Socio- Demographic Characteristics of the Study participants.

1. Age ……..
2. Gender 1) Male 2) Female
3. Education level …….
4. Occupation …….

II. Clinical presentation and associated factors questions.

1. Is there any history of hospitalization in the past 6 months? Yes ☐ No ☐
2. Household member hospitalization in the past 1 year Yes ☐ No ☐
3. Is there any indwelling medical device in the past 1 year? Yes ☐ No ☐
4. Oral antibiotic usage in the past 3 months? Yes ☐ No ☐
5. HIV status Pos. ☐ Neg. ☐
6. Dialysis status in the past 1 year Yes ☐ No ☐
7. Chronic wound in the past one year Yes ☐ No ☐
8. History of Surgery in the past 1 year Yes ☐ No ☐

III. Comments_____

Name of principal investigator _____

Signature _____ Date _____

Annex V: Standard Operative Procedures (SOPs)

5.1 Media Preparation

Media Preparation and Quality Control:

General Guidelines

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

A wide range 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 or demineralized water 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

performance of such media is seriously impaired by subjecting them to heat. For tubed media (except those that require supplements), dispense media before autoclaving.

For quality control (QC) of autoclaving, autoclave tape or paper is placed on the medium flask at the time of autoclaving. Over-sterilizing media can cause a number of problems, including:

- Incorrect pH
- Decrease in the gelling properties of agar
- Development of precipitate that is not typical
- Caramelization or darkening of the medium
- Loss of nutritive value
- Loss of selective or differential properties

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. Warm blood 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

Commercial 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 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

Plated 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. Alternatively, when preparing **selective** media (e.g., MacConkey [MAC], Xylose Lysine Desoxycholate [XLD], Thiosulfate Citrate Bile Salts [TCBS] agar, etc.), conditions are such that there is little chance that the cooling media will be contaminated after the agar is poured into the plates. The lids can be placed on the dish so that a small opening is left to let the heat out, resulting in the formation of less condensation on the upper lid. The lid should remain slightly open like this for approximately 30 minutes while the agar solidifies. If it is likely that the agar will be contaminated when the lid is left partly open, the agar should be allowed to solidify with the lid closed.

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.

Tubed media: Dispense media to appropriate tubes and autoclave with caps loose. Immediately recap tubes to reduce the chance of contamination. Slant tubed media (e.g. TSI) to provide a deep butt (at least 3 cm) and a short slant (2 cm).

- For slants, dispense 4 ml in 16 x 100 mm screw-cap tubes
- For slants and butts, dispense 5-6 ml in 16 x 100 mm screw-cap tubes
- For broths (Alkaline Peptone Water, Selenite), dispense 5-6 ml in 16 x 100 mm screw-cap tubes
- For deionized water and Normal Saline Solution (NSS) dispense 4 ml in 13 x 100 mm or 16 x 100 mm screw-cap tubes

8. Storage of Prepared Media

Media should always be stored under controlled conditions to ensure its quality through to the expiry date.

Label plates/tubes 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, lot/batch number, preparation date, expiration date, and storage temperature (2-8^{o}C).

Most media, especially those containing dyes or indicators, should be protected from light during storage.

For media contained in screw-cap tubes or bottles, tighten the caps, place tubes in a box, and store under appropriate conditions. Label the box with media name, lot/batch number, preparation date, expiry date, and storage temperature (2-8^{o}C or room temperature).

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.

a. Perform QC.
b. Record results on appropriate QC form.
c. Label media bag or box with QC label indicating acceptability of QC results.
d. 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/tube 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 (e.g., Shigella on MacConkey agar).

Testing for ability to produce appropriate biochemical reactions: For selective media, use at least one pathogen and one non-pathogen to test for the medium's ability to differentiate target organisms from competitors (e.g., for MAC, use *E. coli* and *Shigella*).

For biochemical media, use at least one organism that will produce a positive reaction and one organism that will produce a negative reaction (e.g., for urea medium, use *Proteus* and *E. coli*)

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. 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.

5.2 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 culture of the organisms from an 18-24 hour agar plate, preferably a nonselective medium like sheep blood agar.

4. 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.

5. 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 a 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 dessicant. 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

6. 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 nonreflecting 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.

7. Reporting

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

8. Procedural Notes

a. This method applies to susceptibility testing of nonfastidious 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, reidentify and retesr 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.

9. References

a. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – 10th edition. CLSI Document M02 – A10. Clinical Laboratory Standards Institute (CLSI) 940 Wayne, PA, U.S.A. 2009.

b. 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.

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

Annex VI: Laboratory Analysis Result Form

1. Sample Source ; Nasal swab
2. Sample unique ID Number;_____Clients MRN_____
3. Types of culture media used; BAP MSA
4. Culture ;

4.1 culture observations and work-up on BAP: QC result_____

Date	Tech Initial	Observations and work-up

4.2 Culture observations and work-up on MSA: QC result_____

date	tech initial	observations and work-up

5. 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)

6. Biochemical Tests; Catalase; _____

QC date	Lot #	Expiry date	QC Results		QC Passed/Failed
			S. aureus	*S. pyogenes*	

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

Coagulase; _____

QC date	Human frozen plasma			QC Results		QC Passed/Failed
	Lot #	Expiry date	Date received	*S.aureus*	*S.epidermidis*	

QC Organisms and Expected Results: *S. aureus* ATCC 25923 – Positive (clumping/clotting)

S. epidermidis ATCC 12228 – Negative (No clumping/clotting)

7. SUSCEPTIBILITY TEST RESULTS:

Date performed:_____ Tech Initial: _____			
ANTIBIOTIC (Disk abbreviation & content in µg)	QC RESULTS	TEST RESULT	
	QC Passed(√)/Failed(X)	Zone size (mm)	Interpretation (S, I, R)
PENICILLIN (PEN-10)			
CEFOXITIN (CXT-30)			
ERYTHROMYCIN (E-15)			
CLINDAMYCIN (CLN-2)			
COTRIMOZAZOLE (COT-25)			
TETRACYCLINE (TET-30)			
CIPROFLOXACILLINE (CPR-			
CHLORAMPHENICOL (CHL-			
GENTAMICINE (GEN-10)			
VANCOMYCIN MIC			

Test Organism: *S. aureus* 25923 on Mueller Hinton medium. Incubate at 35^{O}C 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 VII: Zone of inhibition interpretation chart for Antimicrobials

Antimicrobial agents	disc content in µg	Interpretive Categories and Zone Diameter Breakpoints in mm		
		Resistance	Intermediate	Susceptible
Penicillin	10	≤28	-----	≥29
Cefoxitin	30	≤ 21	------	≥22
Erythromicin	15	≤13	14-22	≥23
Clindamycin	2	≤14	15-20	≥21
Trimethoprim-Sulphamethoxazol,	1.25/23.75	≤10	11-15	≥16
Tetracycline	30	≤14	15-18	≥19
Ciprofloxacillin	5	≤15	16-20	≥21
Chloramphenicol	30	≤12	13-17	≥18
Gentamicin	10	≤12	13-14	≥15
Vancomycin	MIC	≥16	4-8	≤2

Source (CLSI, 2018).

Annex VIII: Antibiotic sensitivity test

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Akele Kubi (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Kassu Desta (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.