ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 hospital medical college and AABET Hospitals

By: - ANDUALEM GAREDEW

Advisor: - ADANE BITEW (MSc, Ph.D.)

Co-advisors:-ZERIHUN W/SENBET (MSc)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology track).

June/2021

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSTY

SCHOOL OF GRADUATE STUDIES

This is testify that the thesis prepared by Andualem Garedew entitled " Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 hospital medical college and AABET Hospitals" and submitted in parial fulfillment of the requirements for the degree of Master in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Speciality) complies with the regulations of the University and meets the accepted standards with respect to orginality and quality.

Signed by the Examining Committee

External Examiner _____ Signature_____Date_____

Internal Examiner _____ Signature_____Date_____

Advisor _____ Signature_____Date_____

Advisor _____ Signature_____Date_____

_____

Chairman of the Department on Graduate Program Coordinator

Table of Contents

Acknowledgments

First of all, I would like to thank the Almighty God, for His grace and providence. Then I would like to acknowledge the Department of Medical Laboratory Science, Addis Ababa University for giving me this opportunity to prepare my proposal and then conduct this thesis work. I would like to express my deepest gratitude to my advisors Dr. Adane Bitew and Mr. Zerihun W/Senbet for their consistent valuable advice, comments, immediate response and follow up.

I am very thankful to Y12HMC and AABET Hospital burn unit for the sample collection and also Y12HMC laboratory for the provision of materials for the study and arrangement of a conductive working environment in the laboratory and their assistance during laboratory investigation.

I would also like to express my deepest gratitude to my wife Feven Samuel and her sister Miraf Samuel and also our family.

List of tables

List of Figures

Abbreviations

AABET	Addis Ababa Burn and Emergency Trauma Hospital
AST:	Antimicrobial susceptibility testing
ATCC:	American Type Culture Collection
BAP	Blood agar plate
CDC	Centre for disease Control and Prevention
CLSI:	Clinical and Laboratory Standards Institute
DNase	Deoxyribonuclease
DRERC:	Department of research and ethics review committee
HAI	Hospital acquired infection
ISO	International Organization for Standardization
MAC	Mac Conkey agar plate
MDR	Multidrug resistance
MRSA	Methicillin-resistant *Staphylococcus aureus*
NI	Nosocomial infections
SDA	Sabourad dextrose agar
SPSS	Statistical Package for the Social Sciences
TBSA	Total body surface area
WHO:	World Health Organization
Y12HMC	Yekatit 12 Hospital Medical College

Abstract

Background: Managing the burn wound is necessary to heal the wound early but the infected one not only needs appropriate management of burn therapy, in addition to that, doing culture for the potential bacteria and their antimicrobial susceptibility pattern necessary. Furthermore Large wound surface, impaired immune systems, and broad-spectrum antibiotic therapy contribute to the growth of opportunistic fungal species. Mycosis in burns is likely to be underestimated.

Objectives: To determine the magnitude of bacterial and fungal infections and their antimicrobial susceptibility pattern of burn patients at Yekatit12 and AABET Hospital, Addis Ababa, 2021.

Methods: A cross-sectional study was conducted in Y12HMC and AABET hospital from January to June 2021. Burn Wound swab specimen was collected using sterile cotton swab and processed and analyzed for bacteriological and fungal culture medias like Potato dexterous agar, Chrome agar, Blood agar, MacConkey agar, Biochemical test and AST. The data was analyzed using SPSS version 25.

Results: 240 burn patients were included in the study a total of 154(64.2%) bacterial pathogens were recovered. Among the isolated 154 (64.2%) bacteria 110 (71.4 %) were gram-positive and 44 (28.5 %) gram-negative bacteria. From gram-positive bacteria, predominant bacteria isolates were CoNs 55 (22.9 %), followed by *S.aureus* 54 (22.5%) while gram Negative bacteria isolated the most predominant bacteria were *Pseudomonas aeruginosa* 17(7.1%)from 66 (27.5%). Fungus isolation 38 (57.5%) were yeast and 28 (42.2 %) mold .The susceptibility patterns of isolates 60% of resistance to the antibiotics tested. *Klebsiella species* demonstrated high level of resistance to Ampicilin and ceftazidime each 5(100%), Tobramycin, ciprofloxacin and gentamicin each 3(60%). Amikacin was relatively effective against 23(95.8%) of the *Klebsiella* species.

CONCULUSION: The wounds were contaminated with various types of bacteria. This might be a reflection of inappropriate use of antibiotics. The presence of MDR in the wound may be a factor for persisted infection. Some of the participants in the study area had a positive fungal result. It is not contemptible it an alarming finding that needs more focus and follow-up on the fungal infection.

Key words: multidrug resistance, antimicrobial susceptibility pattern.

1. Introduction

1.1 Background

Skin serves the function of controlling the spread of microbial populations that reside on the skin while also ensuring the integrity of underlying tissues and protecting them from pathogens. The exposure of subcutaneous tissue results in a loss of skin integrity, thus eventually providing a warm and conducive environment for microbes (1). The invasion or colonialism of wounds often involves numerous types of differing microorganisms that are likely pathogenic, so any wound could be exposed to infection. Infected wounds are more likely to become barriers to the rate of healing of wounded tissue while also having a regressive impact on a patient's quality of life. Wounds that are infected are most often painful while on top of that posing significant discomfort and inconvenience (2).

Burns are categorized by their complexity and harshness as 1st, 2nd, 3rd, and 4th degree. First-degree burns are typically imperfect to redness, a white sign, and slight discomfort at the site of a wound. These burns involve only the epidermis. Second-degree burns are obvious as erythema with superficial sweltering of the skin, involving the superficial dermis, and can also involve the deep dermis layer. Third-degree burns happen when the epidermis is missing with damage to the subcutaneous tissue. Burn victims will show charring and life-threatening injury of the epidermis, and occasionally firm eschar will be present. Fourth-degree burns damaged muscle, tendon, and ligament tissue, thus resulting in charring and disastrous destruction of the subcutaneous tissue (2).

Immune suppression is triggered in cases where significant burn injuries, this suppression extend to the suppression of immunity (both specific and nonspecific). An imbalance in the normal balance causes a burn wound sepsis and this leads to an increase in number of bacteria. Burn wounds provide a fertile area for the growth and spread of microorganisms that are of exogenous and indigenous origin. The suppressing of immune systems and a prolonged hospital stay are the two major factors that contribute to the development of hospital acquired infection (3). Loss of first line of defense against microbial infection is a major health concern pertaining to burn patients. Patients are affected by the deactivation of vascular tissues, changes in the components (i.e. nonspecific and specific) of the immune system and eventually hospitalization. Hospitals are safe haven for the growth of infections that is made evident in the fact that burn patients only remain sterile for 48 hours before the spread of microbes persist. Hospital acquired infection usually has an incubation period of $\geq$ 48 hours after admission (4).

At first, the burned region is viewed as being rid of major microbial pollution. Notwithstanding, gram-positive microorganisms in the profundities of sweat organs and hair follicles may endure the warmth of the underlying injury, and except if effective antimicrobial specialists are utilized, These are principally gotten from the patient's gastrointestinal and upper respiratory parcels just as they are equally garnered from a clinical environment (4). Aerobic bacterial isolates from burn wounds have ranged from Gram-positive organisms like *Staphylococcus aureus,* coagulasenegative staphylococci and *Enterococcus* species, to Gram-negative organisms like *P.aeruginosa, E.coli, Klebsiella pneumoniae, Enterobacter spp, Proteus spp and Acinetobacter spp* (4, 5).

The worldwide problem of rising antibiotic resistance demonstrates no mark of bettering and the current time has been named the "end of the antibiotic era" (6). Critics have declared that if nothing is done, previous minor infections that were effortlessly treated with a humble of antibiotics, might be in upcoming year they will untreatable as in the days before antibiotics were discovered(7) . Resistance genes arise through natural selection in the atmosphere over a long period of time or by a spontaneous mutation in the microbial DNA. A resistant pattern has been reported by almost all antibiotics that have been developed so far. The infections caused by antimicrobial-resistant microorganisms often fail to respond to the standard treatment or drug therapy, which result in prolonged illness and fatal risk. It has been credited to the extensive obtainability of antibiotics over the pawn, incorrect prescription practices, poor patient obedience leading to discontinuing treatment too early, and overuse of antibiotics in livestock feedstuffs (8).

Multi-drug Resistant (MDR) organism such as Staphylococcus aureus, Pseudomionas aeruginosa are more common in burn patients. Ampicillin, metronidazole, amoxicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, nalidixic acid, gentamicin, and ceftazidime are common antibiotics whose resistance pattern has been elevated in recent years. The rise and dissemination of resistant bacteria have contributed to increasing cases of antimicrobial resistance (7).

In addition, these patients are at high risk of developing fungal infection than those who are admitted for other reasons. Fungi are eukaryotes and they're more intricate compared to bacteria. Although frequently underrated, invasive wound infection due to species like candida as well as other opportunistic ones are principal causes of late-onset morbidity and death in those with major burn and severely disturbed immunity. Major risk factors could be an increase in burned total surface area, impaired immunity, abuse or frequent use of broad spectrum antibiotics which results in elimination of the normal flora and invasion of the opportunistic species (8).

The above mentioned risk factors play a part in the development of opportunistic infection. This will result to a challenging fluid resuscitation, extraction of wound and stabilizing the cardio respiratory system and mycoses are expected to be misjudged. Diagnostic execution can be deferred now and then since clinical signs are nonspecific and isolation of colonization from infection is precarious. Therapeutic measures can range from preventive prophylaxis to treatment with antifungal therapy and radical amputation of infected limbs (9).

The experience accumulated over the past three decades, in the early interventional treatment of burns patients has dramatically changed the cause of death; it is now estimated that about 75% of the mortality following burn injuries is related to infections, rather than osmotic shock and hypovolemia. Therefore, knowledge of the responsible bacterial flora of burn wounds, its prevalence and bacterial resistance and fungus is crucial importance for fast and reliable therapeutic decisions (10).

1.2. Statement of the problem

Burns and burn-related injuries are still a major public wellbeing issue. Fantastically, around 200-300,000 individuals die from fire-related burns each year. Every day, over 30,000 people endure from new burn injury around the world, extremely sufficient to warrant therapeutic consideration, comparing to an assessed 11 million new cases each year universally. The larger parts of these happen in moo and center salary nations and nearly two third happen in African and South-East Asia locales. In developing nations, like Ethiopia, burn wound contaminations are major wellbeing issues (11).

Burn death rates have been declining in many high-income countries, and child burn death rates are currently approximately seven times higher in low- and middle-income countries than in high-income countries. Burn injuries are a common and serious type of harm, with 1 percent of individuals in the globe experiencing a true burn injury at some point in their lives. Burn injuries are also estimated to account for 1% of all illnesses in people who visit a hospital for treatment. Surprisingly, the bulk (>95%) of burn injuries occur in developing countries, resulting in significant misery, disability, and mortality. Infection of burn wounds is a common consequence beyond the initial phase of shock, and it is a major cause of mortality in burn patients. More than 75 percent of burn patients die as a result of infection, and these patients are still at risk of dying unless the burn wound heals completely (12).

Antibiotic use has resulted in the rise of antibiotic-resistant bacterial pathogens in wound infections, resulting in significant morbidity and fatality rates. On the one hand, rising prevalence of multidrug resistant bacterial pathogens complicates hospital acquired infections, while on the other hand; large wound surfaces, weakened immune systems, and broad-spectrum antibiotic therapy promote the establishment of opportunistic fungal species (13).

Wound infection is a major source of concern among healthcare providers, not only because of the increased trauma to the patient, but also because of the financial burden it places on the system and the growing demand for cost-effective management. The ability to identify the causative agents of wound infection has proven useful in the selection of empirical therapy, infection control measures in health facilities, and the formulation of antibiotic policy rationales (14).

Because of the health risks associated with burn wound infection and treatment resistance, it's critical to keep track of bacterial and fungal pathogens as well as their drug susceptibility. The extensive practices of antibiotics, composed with the extent of the period over which they have been obtainable have led to main problems of resistant organisms contributing to morbidity and mortality. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. Understanding the causative

agents of wound infection has proven to be helpful in the selection of empiric antimicrobial therapy, and it decreases the bed occupancy rate in the hospital. According to one study in Ethiopia, the bacterial profile among burn patients was 83 percent, which is a very high percentage. However, studies on this topic are scarce; in the last decade, only one study has been undertaken at Yekatit 12 Hospital Medical College, and no study has been conducted at Aabet Hospital. Our country's studies are all focused on bacteria; there isn't a single study that teaches us about fungus infections. However, in this study, the fungus infection will be isolated on SDA. The type of pathogenic bacteria species and their AST will also be looked into. The goal of this study is to investigate the magnitude of bacterial and fungal infections, as well as their antibiotic susceptibility patterns, in burn patients at Yekatit12 and AABET Hospital in Addis Ababa, Ethiopia, in 2021.

1.3. Significance of the study

Burn wound infections due to bacterial and fungal is foremost roots of illness and demise in Ethiopia. And this increases bed occupies rate and hospital stay time of a patient. Most patients treated empirically. Therefore, knowing and understand the etiology of major pathogens causing infections and their antibacterial susceptibilities may play a positive role in better wound management. For these reasons, surveillance burns wound infection by doing culture and antibiotic resistance patterns initial. The result of this study could help provide information on the magnitude of multidrug resistance pattern infection-causing bacteria. Helps physician to select better antibiotic, Help patient to get appropriate treatment and finally it will be a baseline for another researcher to do prospective studies for the future because it assesses the current event.

2. Literature Review

Many studies have demonstrated the ease with which pathogenic microbes can be infected patients. Different studies in various parts of the world had assessed the extents of bacterial and fungal infection. For example, a cross sectional studies in Bacterial Infections and Antimicrobial Resistance Patterns of Burn Wound Infections: A One Year Study from Burn Hospital, Isfahan, Iran was conducted. From the total of 1500 wound culture, 957(63.8%) samples were detected as positive. The most common gram-negative bacteria were *Acinetobacter baumannii* (34.9%) with the highest and the lowest antibiotic resistance to Ceftazidime and Tobramycin, respectively. Among recovered Gram-positive isolates, *Staphylococcus aureus* (10.2%) were the predominant isolates with the highest and the lowest antibiotic resistance to Penicillin and Vancomycin, respectively (14). Another cross-sectional study was carried in the same country showed that P. *aeruginosa* was the leading Couse of infection and *Acinetobacte*r has appeared as an emerging pathogen (15). Other study in this country by Ezzatollah Rezaei et al *Pseudomonas aeruginosa, Acinetobacter* and *Klebsiella* were the most common Gram-negative and *Staphylococcus aureus* was the most common Gram-positive organisms recovered from the patients (16).

A retrospective study was conducted in the burn unit of the Govt. Medical College Hospital, Chandigarh, India. *Pseudomonas aeruginosa* was found to be most common isolate (59%) followed by *Staphylococcus aureus* (17.9%), *Acinetobacter* spp. (7.2%), *Klebsiella* spp. (3.9%), *Enterobacter* spp. (3.9%), *Proteus* spp. (3.3%) and others (4.8%) and Amikacin was found to be the most effective drug against gram negative bacteria, For *S. aureus* and *P. aeruginosa* netilmicin and piperacillin were found to be the most effective drugs. Most of the isolates showed high level resistance to antimicrobial agents in this research (9). Another retrospective analysis of wound swabs of patients admitted the similar hospital and country the isolate was *Pseudomonas species* (spp.) in 54.16%, followed by *Staphylococcus aureus* (20.83%), *Klebsiella spp*. (3.57%), *Eseherichia coli* (2.67%) and *Proteus spp.* (2.67%). Other isolates 12.50%) included *Acinetobacter* spp., *Enterococci* and coagulase-negative *Staphylococci12*.50%) (17).

Other study in India in antibiotic resistance of burn patients shows all *Staphylococcus aureus* isolates were sensitive to Vancomycin (100%), followed by Doxycycline (67%) and Amikacin (62%). Most of the *Klebsiella pneumoniae* isolates were resistant to third generation Cephalosporins (62%) but sensitive to Imipenem (100%), Levofloxacin (100%), followed by Piperacillin and Tazobactam (87%), amikacin(51%). Imipenem (100%), Levofloxacin (100%), followed by Piperacillin and Tazobactam (98%) were sensitive in most of the isolates of *Pseudomonas aeruginosa* (18).

Changing trends in bacteriology of burns in the burns unit, Delhi, India shows *Pseudomonas* species (31%) and *Staphylococcus aureus* (22%) were the most common pathogens followed by *Klebsiella species* (19%). Multi-drug resistant (MDR) *Acinetobacter species* (9%) (19).

Further study in India *Pseudomonas aeruginosa* (42.9%) and *Staphylococcus aureus* (24.3%) were the most common organisms followed by *Staphylococcus epidermidis* (15.7%), *Proteus* spp. (8.6%), E.coli (5.7%) and *Klebsiella pneumoniae* (2.8%). *Pseudomonas aeruginosa*, *E.coli* and *Klebsiella pneumoniae* were found to be highly resistant to commonly used antibiotics. High rate of multiple antibiotic resistances was observed in both Gram positive and Gram negative bacterial species recovered (20). Bacterial and fungal profile of burn wound infections in Tertiary Care Center in India isolated was *Klebsiella pneumoniae* (34.40%) followed by *Pseudomonas aeruginosa* (23.94%), *Staphylococcus aureus* (22.94%), *Escherichia coli* (7.34%), *Acinetobacter* spp. (2.75%), *Proteus mirabilis* (2.75%), and *Citrobacter* species (1.38%). *Candida* species (4.59%) was the only fungus isolated, of which *Candida albicans* (50%) was the most common. Gram-negative bacteria were the most sensitive to imipenem (93.67%) and amikacin (75.94%) while Gram-positive bacteria were the most sensitive to linezolid (100%) and vancomycin (100%) (21)

Another study in Far East Asia Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea .*Pseudomonas aeruginosa* was the most common (45.7%) isolate from the burn patients followed by *Staphylococcus aureus* (19.2%) and *Acinetobacter baumannii* (13.4%) (22).

From Latin America in brazil there is another prospective study of fungal and bacterial flora of burn wounds was carried out at the Burns Unit of Hospital Regional da Asa Norte, Brasília, Brazil Single the most common isolate overall was *S. aureus* (20.5%) followed by coagulase-nagative *staphylococci* (15.2%), *P. aeruginosa* (11.4%), *Klebsiella sp*. (11.2%), and *Enterobacter sp*. (10.4%) (23). other study from Brazil for *S. aureus* and *P. aeruginosa* vancomycin and polymyxin were found to be the most effective drugs. Most of the isolates showed high level resistance to antimicrobial agents. *Candida tropicalis* was the most predominant Fungi, followed by *Candida parapsilosis* (24).

Several authors have demonstrated that burn wound microbial colonization, and antimicrobial profiles are Key risk factors for infections. In Europe from Italy the most common bacterial species detected was *Staphylococcus aureus* (37%), followed by *Pseudomonas aeruginosa* (17%), *Proteus mirabilis* (10%), *Escherichia coli* (6%) and *Corynebacterium* spp. (5%). Polymicrobial infection was found in 59 (27·1%) of the samples and was mainly constituted with two species. The most common association was S. aureus/P. aeruginosa. All Gram-positives were susceptible to vancomycin and linezolid. Gram-negatives showed quite high resistance to the majority of antibiotics, being amikacin the most active against these bacteria (25)

Other studies from the same continent in this time from Turkey their results revealed that the most frequent isolate was *Acinetobacter baumannii* (23.6%), *Pseudomonas aeruginosa* (12%), *Staphylococcus aureus* (11.2%), *Escherichia coli* (10%) respectively. Multidrug-resistance has emerged as an important concern in burn units. Tigecycline, and colistin were found to be the most active drugs against *Acinetobacter*

baumannii. Carbapenems and amikacin, were found to be the most active drugs against other gram negative bacteria. Vancomycin and linezolid were active against gram positive bacteria (4).

In our continent also there are many study Colonization of burn wounds in Ain Shams University Burn in Egypt Unit. the most frequent isolate was *Pseudomonas aeruginosa* (21.6%), followed by *Klebsiella pneumoniae* (15.2%), then *Escherichia coli* (13.6%), *Staphylococcus aureus* (13.2%), coagulase-negative *Staphylococci* (11.6%), *Streptococcus pyogenes* (8.3%), *Enterobacter* species (6.6%), and lastly *Streptococcus faecalis* and *Candida albicans* (5.9 and 3.6%, respectively) (14).Other study from the same country found that pseudomonas (49%) was most isolate bacteria. Multidrug resistant gram negative organisms represent about 60% of the isolates (26).

A *Staphylococcus aureus* was the predominant microorganism (25%) followed by Escherichia coli (12%), Pseudomonas aeruginosa (9%) and *Staphylococcus epidermidis* (9%) (27) A Retrospective, descriptive study was conducted in Mthatha, Eastern Cape, South Africa by V G Bhat & S D Vasaikar. The commonest organism was S. aureus (27.7%), followed by K. pneumoniae (13.4%), *Proteus mirabilis* (12.4%), Group D *streptococcus* (9.4%), *P. aeruginosa* (8.9%) and *E. coli* (6.2%). Methicillin-resistant *S. aureus* accounted for 57.5% of the *S. aureus.* Resistance among the Gram-negative bacilli was, in general, least to imipenem, amikacin and ciprofloxacin (28).

In our county there are some studies, at jimma south west Ethiopia cross sectional study was conducted by Mohammedaman Mama, et.al from 150 specimens showing an isolation rate of 87.3%. The predominant bacteria isolated from the infected wounds were *Staphylococcus aureus* 47 (32.4%) followed by *Escherichia coli* 29 (20%), *Proteus* species 23 (16%), Coagulase negative *Staphylococci* 21 (14.5%), *Klebsiella pneumoniae* 14 (10%) and *Pseudomonas aeruginosa* 11 (8%). All isolates showed high frequency of resistance to ampicillin, penicillin, cephalothin and tetracycline (29). Another studies Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Referral Hospital, Northwest Ethiopia A total 9 of 137 study subjects were included in the study with bacterial isolation rate of 115 (83.9%). Seventy-seven (57%) of the isolates were Gram-negative and 59 (43%) were Gram-positive. From the total isolates, *Staphylococcus aureus* was the most predominant isolate (34%) followed by *Klebsiella* species (13%), coagulase negative *staphylococci* spp. (12%) and *Pseudomonas aeruginosa*. Gram positive isolates were resistant to ampicillin (86.4%), amoxicillin (83%), penicillin (81.3%), oxacillin (74.6%), and tetracycline (59.4%), while Gram-negative isolates were resistant to amoxicillin (97.4%), ampicillin (94.8%), tetracycline (72.7%), trimethoprim/sulfamethoxazole (66%), and chloramphenicol (54.5%) (30).

A cross sectional study was conducted at Yekatit 12 Hospital medical college Burn unit, *S. aureus,* (34.04%), and P. aeruginosa, (31.8%), were predominant. Antimicrobial resistance was observed for

Ampicillin, (77.4%), Doxycycline, (74.0), Nalidixic acid, (70.5%), Penicillin G, (68.2%), and tetracycline, (67.5%) (31). Another Cross-sectional, prospective study conducted from March to May 2011 in yekatit 12 hospital medical college. Out of 114 patients, bacterial infection was observed in 95(83.3%) of which, 66 (69.5%) had *S. aureus* infection. Overall prevalence of *S. aureus* isolation was 57.8%. Most of them were sensitive to vancomycin, clindamycin, Kanamycin and Erythromycin, but highly resistant to penicillin (32).

3. Objectives

3.1 General objective

To assess and determine the magnitude of the bacterial and fungal infections and their antimicrobial susceptibility pattern of burn patients at Yekatit12 and AABET Hospital, Addis Ababa, 2021.

3.2 Specific objectives

- To determine the profile of bacteria recovered from burn wound infection
- To determine the presence of fungus from burn wound infection.
- To determine drug susceptibly profile of bacterial from burn would infection.

4. Materials and Methods

4.1 Study area

The study was conducted in Addis Ababa city Administration, the capital city of Ethiopia. The study site is Yekatit 12 Hospital medical college (Y12HMC). The Y12HMC serves as a referral center for all medical conditions from the surrounding hospitals, health center and clinics from all regions. The burn unit consists of one room with 7 beds for pediatrics, and Adult burn unit consists of two rooms with 18 beds.

The other study area is Addis Ababa Burn, Emergency & Trauma Hospital (AABET Hospital) is an affiliate of St. Paul's Hospital Millennium Medical College and is inclusive of four major departments; Emergency Medicine and Critical Care, Plastic Reconstructive and Hand Surgery, Orthopedics & Traumatology, and Neurosurgery. The burn unit consists of one room with 17 beds for pediatrics, and Adult. Both hospitals laboratory is well equipped to handle blood, urine and stool samples in a hematology, clinical chemistry. Microbiology units are only available in Y12HMC.

4.2 Study Design and period

Hospital based Prospective cross sectional study was conducted from January to June 2021.

4.3 Population

4.3.1 Source population

The source population was all burn wound patients admitted at Y12HMC & AABET in the study period.

4.3.2 Study population

The study population was all burn wound patients who full fill the inclusion criteria.

4.4 Inclusion and Exclusion criteria

4.4.1. Inclusion criteria

1. Any burn wound patients attending at burn Unit in Y12HMC & AABET hospital after 48 hour of admission.
2. Patient who will be Volunteer to give informed consent/ ascent to participate in the study.

4.4.2 Exclusion criteria

- Who have no visible wound and heal wounds.

4.5 Study Variables

4.5.1 Dependent Variables

- Bacterial profile
- Fungal profile
- Antimicrobial sensitivity test

4.5.2 Independent Variables

Socio demographic factors: age, residence, marital status, educational and occupational status.

- Hospital stay

4.6 Measurement and data collection

4.6.1 Sample size calculation

All burn patients were included in the study until the required sample size is obtained. Since the study is based on a single population proportion, the sample is calculated as follows;

$$n = \frac{Z^2_{1-/2}\, P\,(1-P)}{d2}$$

Where;

N is the sample size to be determined, Za/2 is 1.96

Sample size is determined using single population proportion formula with inputs of 95% confidence level,

Where: n = Sample size, p = burn infection S (83.3%), q = (1-p),

d = tolerated margin of error $(0.005)^2$

$\frac{(1.96) \times 0.833 \times 0.167}{(0.05)^2}$ = **214,** with addition of 10% of non-respondent rate it will be **235.**

4.6.2 Sampling method

Hospital based Prospective cross sectional study was conducted. Conventional sampling technique was employed to include study participants who meet the inclusion criteria until the achievement of the sample size. The sample site was allocated to selected hospital proportional to their case flow. Total patients admitted in the last year are 720 in both Hospitals. Out of the total number 480 are admitted in Yekatit hospital the other 240 are admitted in AABET hospital. Totally we collected 240 samples from both hospitals out of this 160 are collected from Yekatit and the other 80 are collected from AABET Hospital.

4.6.3 Data collection procedure

Data was collected using structured data collection form to obtain information on socio demographic status, antibiotic usage and detailed clinical history like degree of burn, TBSA and cause of burn. Informed consent was taken from each patient and informed assent was taken on behalf of children from their parents or guardians. (See more on annex 1-8). To avoid double enumeration the client card number was used and after data is collected code will be given to the client card. Before actual data collection, questionnaires were pre-tested by taking 20 burn patients at Yekatit 12 Hospital medical college other than the actual study participant's.

4.7. Laboratory analysis

4.7.1 Sample collection and processing

Study participants were recruited into the study as they admitted to the hospitals burn unit. After 48 hours of admission Open wound swabs were aseptically obtained after dressed wounds were cleansed with sterile normal saline after removing the dressing. The specimen was collected on sterile cotton swab by rotating with sufficient pressure. Double wound swabs were taken from each wound at a point in time to reduce the chance of contamination. The samples were transported to the laboratory after collection using Amies transport media in triple packaging. And Process in Yekatit 12 hospital medical college Microbiology unit for processing. Upon receipt of the sample was inoculated on Blood agar, MacConkey and Sabourd deoxteros agar. All inoculated plats were incubated at the appropriate temperature and day. Each culture plate was examined bacterial and fungal growth.

4.7.2 Laboratory Analysis

4.7.2.1. Culture and identification

Before the identification of the organism Isolation is takes for isolation of bacteria and fungus A Gram stain helps with the visualization of bacteria and fungus and gives an indication of the type of bacteria and fungus present, based on the shape of the bacteria and fungus and the staining properties of bacteria (Gram positive:

purple; Gram negative: pink/red). A Gram stain also helps to identify mixtures of bacteria, helps to determine the appropriate range of agar plates to be used, for fungus isolation we only use Sabouraud dextrose agar plate.

4.7.2.1.1 Identification of bacteria

Once a bacteria has been obtained in pure culture, it has been identified so far identification colonies features are important to differentiate by shape and size .other characteristic are color, odor, the degree of growth and the degree of hemolysis in blood agar. And also fermentation and other biochemical properties like citrate utilization, catalase production, oxidase reaction, sugar fermentation, and indole production.

4.7.2.1.2 Mold Identification

Mycelia fungi were identified by studying their microscopic and macroscopic characteristics. Pigmentation of the front and the reverse side and rate of growth of each culture was considered for macroscopic identification (32).

4.7.2.1.3 Yeast Identification

Yeasts were identified by inoculation on CHROM agar Candida culture medium as per the instruction of the manufacturer (32).

4.7.2.1.4 Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed by Kirby-Bauer Disk diffusion as shown below will two to three pure colonies was picked from original culture plates and mix with test tube containing saline until it become equivalent to McFarland 0.5 standards. Then the bacterial suspension was streaked in the medium by using cotton swap. After, the inoculum (3 to 5min) disks like Vancomycin, Gentamicin,Cefoxitin ,Amikacin and Meropenam was placed on Mueller Hinton agar plates The agar using sterile forceps. Plates were incubated aerobic condition for 24hrs at 35°C. After, overnight incubation the zone diameter was measured with ruler undersurface of the petridish. The susceptibility and resistance was interpreted according to CLSI guidelines 2019.

4.7.2.2 Quality Assurance

The prepared culture media and the sterility of all material were checked. The sterility of culture media was checked by incubating each batch of the prepared media at 37^0Cfor 24 hrs. And observe the presence of growth. Abilities of all prepared media supporting growth was also be checked by inoculating known strain based on the type of media The sample tube will be label with the same unique survey identification number (USIN) Control strain organisms such as Gram Positive *S.aureus* (ATCC 25923), *E.coli* (ATCC 25922)*P.aeruginosa (ATCC 27853)* was used to test the performance of prepared culture Medias and the efficacy of the antibiotics agents. Standard operating procedures will follow strictly in every step of the

work. The functionality of instruments was check before use. Unexpired media use and Manufacturer`s instructions regarding media preparation are follow.

4.8 Data Quality Assurance

The questionnaire were prepared first in English and translated into the local language (Amharic). The translated Amharic version will be pre-tested on burn patient prior to the actual survey and modifications will be made accordingly. Training will be given for data collectors and supervisor to have consensus and the same understanding of what is intended to be measured by each question in the questionnaire. The questioner will be assessed before the actual data collection .Every activity in the laboratory will be done by adherence with standard operation Procedures. The specimen will be kept free of contamination. All materials, equipment and procedures will be adequately controlled. Culture media will be tested for sterility and performance. The performance of equipment's (autoclave, incubators) was monitored by using standard procedures. The data was check for completeness and representativeness prior to entry.

4.8.1 Pre analytical phase

Socio-demographic characteristics of patients were collected using structure data collection sheets after getting informed consent. All wound swab culture specimens were collected by well-trained nurse by following standard operational procedure. When specimens reach the laboratory, it was checked to ensure that the correct specimen had been sent and the name on the specimen is the same as that on data collection form. To avoid sample contamination leak proof and sterile sample container was used.

4.8.2 Analytical phase

All materials, equipment and Procedures were adequately controlled. All stain and reagent were clearly labeled, dated and stored correctly. For each item of equipment there is clear operating and cleaning instructions, and service sheets. The temperature of a refrigerator, incubator, and water-bath was monitor and documented culture media was tested performance and sterility.

4.9 Data Analysis and interpretation

Data entry and analysis, SPSS version23 statistical software was used. Overall socio-demographic, clinical characteristics and specific prevalence were calculated using descriptive statistics of the sample through frequencies and cross-tabulations. Finally, the results were presented in words, charts, graphs, and tables. The odds ratio was used to determine association at 95% confidence intervals (CI) and $P < 0.05$. Hence crude odds ratio of infections with the independent variables was calculated using logistic regression analysis. Finally, the results were presented in words, charts, graphs, and tables**.**

4.10 Ethical consideration

Before starting the study, ethical clearance was be obtained from the Departmental Research and Ethics Review Committee of Addis Ababa University College of Health Sciences, School of Sciences, Department of Medical Laboratory Sciences. Then a letter informing to Yekatit 12 Hospital Medical College and AABET Hospital permission was obtained from both hospital to access data from the study population. All eligible subjects were informed as their participation was voluntary and Information obtained at any course of the study was kept confidential. For children, less than 18 the study aim was explained to all mothers/guardians and then Informed assent was obtained from each child's mother/guardian, after explaining the research work, its confidentiality, protection, and anonymity of data. Positive results were made available to clinicians for decision-making as early as available.

4.11 Dissemination of results

After conducting the research, the results of the study will be submitted to Addis Ababa University, College of Health Sciences, and Department of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material will be given to Addis Ababa Health Office, Yekatit 12 Hospital, Aabet hospital annual conferences of professional societies and other concerned bodies. The finding of the study will also be presented to the medical scientific community and manuscript will be submitted to peer reviewed journals for publication.

4.12 Operational definition

Gram-positive bacteria have a thick layer of peptidoglycan in their cell wall. The thick layer of peptidoglycan stains blue or purple after being exposed to a crystal violet dye.

Gram-negative bacteria don't have a thick layer of peptidoglycan in their cell wall. When they are stained with a crystal violet dye their cell walls are unable to retain the color of the dye and instead turn red or pink.

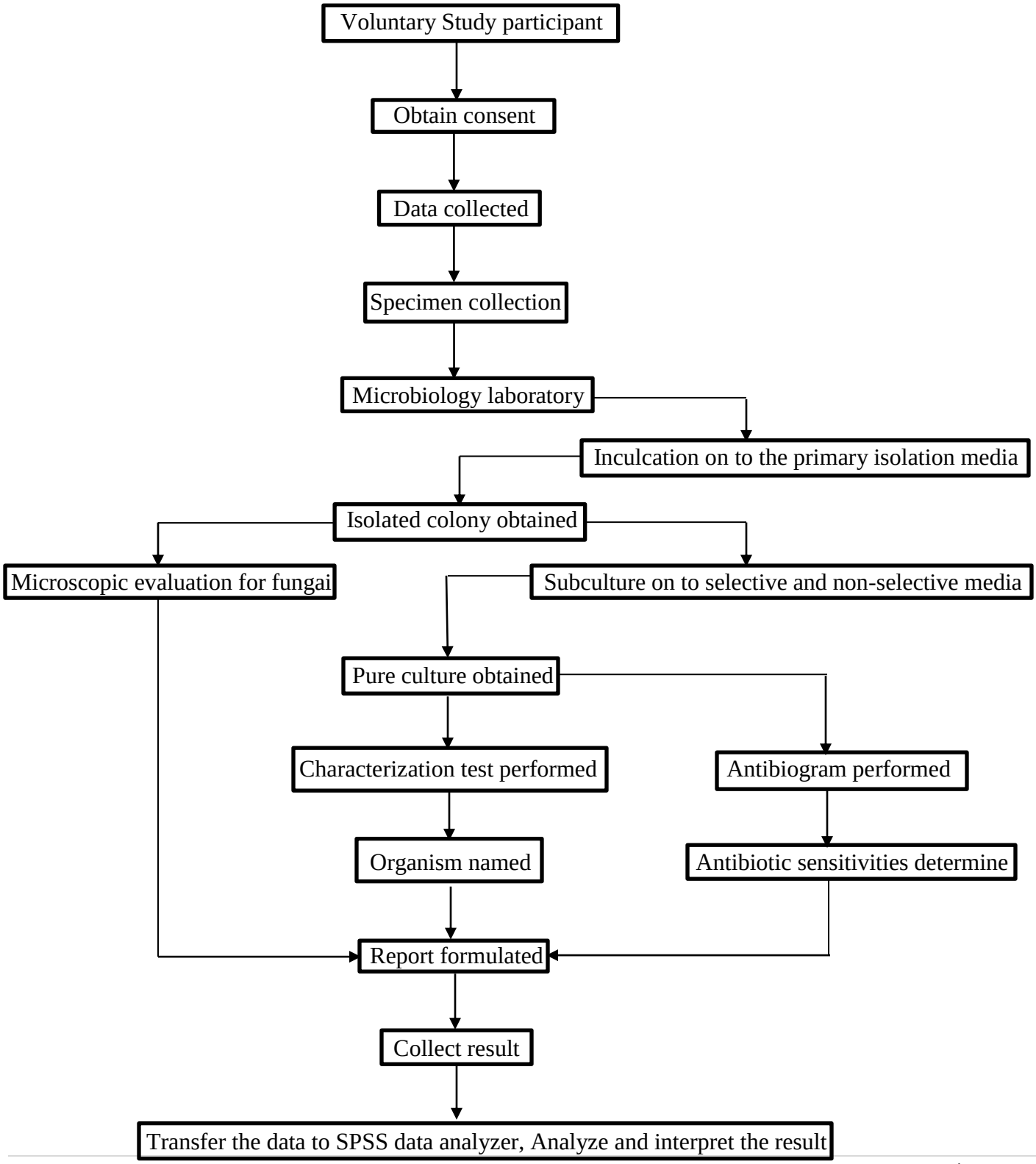
Fungi have complex eukaryotic cells classified in their own kingdom.

Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to.

5. Work Flow

Fig1. Workflow chart

The study was conducted as follows:



6. Result

6.1 Socio-demographic characteristics of study subjects

During the study period, 240 study participants were included in the study with a 100 % response rate. Among them 124 (51.7 %) were male and 116 (48.3%) female. The study participants had age ranges between 1 and 85 years with a mean age of 26years. The majority of the participants was within the age range of 1-14 (30 %) and employed 58(24.2 %). Out of 240 participants, 125(51.2%) were urban residents and the rest 57 (23.8 %) were rural dwellers (Table 1).

Table-1: Demographics of the study population in relation to prevalence of burn wound infection, patients visited Y12HMC & AABET January to June.

Variable		Positive for bacteria No (%)	negative for bacteria No (%)	Positive for fungal	Negative for fungal	Positive for bacteria and fungus
Age in year	1-14	74 (30.3%)	43 (17.3%)	19 (7.9%)	98 (40.8%)	19 (7.9%)
	15-24	32 (13.3%)	6 (2.5%)	11 (4.6%)	27 (11.3%)	8 (3.3%)
	25-44	38 (15.8%)	8 (3.3%)	15 (6.3%)	31 (12.9%)	13 (5.9%)
	45-65	17 (7.5%)	1 (0.4%)	8 (3.3%)	10 (4.2%)	8 (3.3%)
	≥=66	19 (7.9%)	2 (0.8%)	13 (5.4%)	8 (3.3%)	12 (5.0%)
Sex	Male	88 (36.7%)	36 (15.0%)	30 (12.5%)	94 (39.2%)	29 (12.1%)
	Female	92 (38.3%)	24 (10.0%)	36 (15.0%)	80 (33.3%)	31 (12.9%)
Place of residence	Urban	123(51.2%)	38(15.8%)	32(13.3%)	129(53.8%)	31(12.9%)
	Rural	57(23.8%)	22 (9.2%)	34(14.2%)	45(18.8%)	29(12.1%)
Education status	Informal	15(6.3%)	2 (0.8%)	12(5.0%)	5(2.1%)	10(4.2%)
	Primary	48(20.0%)	9 (3.8%)	17(7.1%)	40(16.7%)	15(6.3%)
	Secondary	32(13.3%)	5(2.1%)	15(6.3%)	22(9.2%)	13(5.4%)
	College and above	27(11.3%)	3(1.3%)	9(3.8%)	21(8.8%)	9(3.8%)
	Under Age	58(24.2%)	41(17.1%)	13(5.4%)	86(35.8%)	13(5.4%)
Marital status	Married	41(17.5%)	7(2.9%)	20(8.3%)	28(11.7%)	18(7.5%)
	Single	70(29.2%)	11(4.6%)	23(5.6%)	58(24.2%)	20(8.3%)
	Divorced	5(2.1%)	0(0%)	4(1.7%)	1(0.4%)	4(1.7%)
	Widowed	4(1.7%)	1(0.4%)	4(1.7%)	1(0.4%)	3(1.3%)
	Separated	1(0.4%)	1(0.4%)	1(0.4%)	1(0.4%)	1(0.4%)
	Under Age	58(24.6%)	40(16.7%)	14(5.8%)	85(35.4)	14(5.8%)
Employment status	Employed	58(24.2%)	7(2.9%)	30(12.5%)	35(14.6%)	29(12.1%)
	Not Employed	8(3.3%)	3(1.3%)	2(0.8%)	9(3.8%)	0(0%)
	Student	51(21.3%)	8(3.3%)	16(6.7%)	43(17.9%)	14(5.8%)
	Retired	4(1.7%)	1(0.4%)	4(1.7%)	1(0.4%)	3(1.3%)
	Under Age	59(24.6%)	41(17.1%)	14(5.8%)	86(35.8)	14(5.8%)

6.2 Prevalence of gram positive bacteria and gram negative bacteria

Among the isolated 154 (64.2%) bacteria 110 (71.4 %) were gram Positive and 44 (28.5 %) gram positive bacteria respectively as shown Table 2. From gram positive bacteria predominant bacteria isolates were CoNs 55 (22.9 %), followed *S.aureus* 54 (22.5%) while gram Negative bacteria isolated the most predominant bacteria was *Pseudomonas aeruginosa* 17(7.1%).and followed by *Proteus* spp 8 (3.3 %), *Acintobacter spp* 7 *(*2.9 %), *Klebsiella* 5 (2.1%).The frequency of bacterial isolation was higher in the age group at 1-14 74(30.3%) followed by the age group 25 -44 38(15.8%)

Table-2: Distribution of Gram positive and Gram negative bacteria isolated and other finding from burn wound

	Isolate	Frequency (%)
Gram positive	*S.aureus*	54 (22.5%)
	Cons	55(22.9%)
	B-heamoloytic streptoccus	1(0.4%)
Gram negative		
	P.aeruginosa	17(7.1%)
	Proteus spp.	8(3.3%)
	Acintobacter	7(2.9%)
	Klebsiella spp.	5(2.1%)
Gram positive & Gram negative	*S.aureus & P.aeruginosa*	7(2.9%)
Other finding	Mixed growth	26(10.8)
	No growth	60(25)

6.3 Prevalence of fungal

Among the isolated fungus 38 (57.5%) were yeast and 28 (42.2 %) mold respectively as shown Table 3. From Yeast cells predominant yeast isolates were *C.tropical* 18 (47.3%), followed *C.albican* 13(34.2%) while Molds isolated the most predominant mold was *pencillium* spp7 *(25%)* and followed by *Aspergillus* spp 6 (21.4%) .The frequency of fungal isolation was higher in the age group at 1-14 followed by the age group 25-44(%)

.

Table-3: Prevalence of fungal in age group.

Variable		No fungal growth No (%)	Yeast growth No (%)	Mold Growth
Age in year	1-14	105 (43.8%)	13 (5.4%)	10 (4.2%)
	15-24	35 (14.6%)	9 (3.8%)	5 (2.1%)
	25-44	27 (11.3%)	12 (5.0%)	5 (2.1%)
	45-65	6(2.5%)	4 (0.4%)	5 (2.1%)
	≥=66	1 (0.1%)	0 (0.8%)	3 (1.3%)

Age classification based on WHO classification for health

6.4 Prevalence of bacterial and fungal in both sample collection site

A total of 154 bacterial pathogens were recovered from all specimens processed during the study. Among these, 109 (68.1%) were Y12HMC and 72 (90%) from AABET. Among this Gram positive, Coagulase negative Staphylococci were predominant followed by *S.aureus* in both hospital. (Table 4)

Table-4: Prevalence of bacterial in both sample collection site

Sample collection site	*S.aureus*	*Cons*	*B-heamolytic streptococcus*	*P.aerugi*	*S.aureus P.aerugi nosa*	*Proteus spp.*	*Acintobacter*	*Klebsiella spp.*	Mixed growth	No growth
Y12HMC	41	42	1	10	6	4	5	4	14	33
AABET	13	13	0	7	1	4	2	1	12	27

From 66(27.5%) fungal growth 32(48.8%) from Y12HMC and 34 (52.1%) from AABET. among this yeast were the predominant.

Table-5: Percentage of fungal and bacterial dual infection

fungal and bacterial dual infection	60(25%)
Other growth and non-growth	180(75%)
Total	240(100%)

Table-6: Percentage of fungal cultures in both sample collection sites

Isolated fungi	Y12HMC	AABET
No fungal growth	128 (80%)	46 (57.5%)
C.albican	8 (5%)	5 (6.2%)
C.tropical	8 (5%)	10 (12.5%)
C.krusei	5 (3.1%)	2 (2.5%)
Alternaria	1 (0.6%)	1 (1.2%)
Cladosporium and Pencillium Spp	1 (0.6%)	1 (1.2%)
Cladosporium	1 (0.6%)	0 (0%)
Pencillium Spp	1 (0.6%)	5 (6.2%)
Rholotorula	1 (0.6%)	1 (1.2%)
Mucor	1 (0.6%)	3 (3.7%)
A.niger	1 (0.6%)	2 (2.5%)
Aureobasidium	1 (0.6%)	1 (1.2%)
Fusarium	2 (0.8%)	1 (1.2%)
Aspergillus spp	1 (1.2%)	2 (2.5%)

6.5 Antimicrobial susceptibility pattern

The susceptibility patterns of isolates revealed varying degrees of resistance to the antibiotics tested. Gram negative rods especially *Klebsiella species* isolated from burned wound were highly resistant to most of the antibiotics tested (Table- 7). *Klebsiella species* demonstrated high level of resistance to Ampicilin and ceftazidime each 5(100%), Tobramycin, ciprofloxacin and gentamicin each 3(60%). Amikacin were relatively effective against 23(95.8%) of the *Klebsiella* species. As indicated in Table-7, 60% of *Acintobacter spp* Showed 100% sensitive to amikacin, meropenem, tobramycin and gentamicin. Trimethoprim Sulpamethoxazole Ciprofloxacin and Cefepime were less effective against *Acintobacter spp*. *Pseudomonas aeruginosa* showed 3(12%) resistance to Ciprofloxacin.Amikacin was effective against *Pseudomonas aeruginosa*. Similarly, meropenem, gentamicin, ceftazidime and cefepime each were effective against *Pseudomonas aeruginosa*. *Proteus* species were 100% susceptible to cefepime and clindamycin.

Table-7: Antibiotics susceptibility profiles of gram negative isolates from both hospitals.

Typeof antibiotic	Antibiotic susceptibility pattern of *P.aeruginosa*			Antibiotic susceptibility Pattern *Acintobacter spp*			Antibiotic Susceptibility Pattern *Klebsilla spp*			Antibiotic Susceptibility Pattern *Proteus spp*		
	S %	I %	R %	S %	I %	R %	S %	I %	R %	S %	I %	R %
Ceftazidime (30 µg)	4 (16.6%)	8 (33.3%)	12 (50.6%)	7 (100%)	0(0%)	0(0%)	0 (0%)	0 (0%)	5 (100%)			
Gentamicin (10 µg)	12 (50.7%)	9 (37.7%)	3(12.2%)	7 (100%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (100%)			
Tobramycin (10 µg)	15(62.5%)	8(33.3%)	1(4.1%)	6 (85.7%)	1 (14.2%)	0 (0%)	0(0%)	0(0%)	5(100%)			
Amikacin (30 µg)	18 (75.0%)	6 (25%)	0(0%)	7(100%)	0 (0%)	0 (0%)	5(100%)	0(0%)	0(0%)			
Meropenam (10 µg)	18 (75.0%)	6 (25%)	0(0%)	7(100%)	0(0%)	0(0%)	2(40%)	3(60%)	0(0%)			
Ciprofloxacin (5 µg)	16(66.6%)	5(20.5%)	3(12.5%)	6(90%)	1(10%)	0(0%)	0(0%)	0(0%)	5(100%)			
Cefepime (30 µg)	10(41.6%)	8(33.3%)	6(25%)	5(69.4%)	2(28.5%)	0(0%)	0(0%)	2(40%)	3(60%)	8(100%)	0(0%)	0(0%)
Trimethoprim Sulpamethoxazole (25 µg)				5(69.4%)	2(28.5%)	0(0%)	0(0%)	3(60%)	2(40%)			
Ampicillin(10 µg)							0(0%)	0(0%)	5(100%)	0(0%)	0(0%)	8(100%)
Chloraampheni col(30 µg)							0(0%)	0(0%)	5(100%)	3(%37.5)	4(50%)	1(12.5%)
Erythromycin (15 µg)										3(37.5%)	3(50%)	1(12.5%)
Clindamycin (2 µg)										8(100%)	0(0%)	0(0%)

The major isolate *S.aureus* were resistant to pencillin (100%) and erythromycin (82.9%), Cefoxitin, 48(78.6%) Trimethoprim sulpamethoxazole (60.6%) were relatively effective against S. aureus. *B-heamoloytic streptoccus* were susceptible to all the antibiotics tested. The overall drug susceptibility profile of Gram positive bacteria against antimicrobial drugs tested were summarized under Table 8.

Table-8: Antibiotics susceptibility profiles of gram positive isolates from both hospitals.

Type of antibiotic	Antibiotic susceptibility pattern of *S.aureus*			Antibiotic susceptibility pattern of *β.Hemolytic group*		
	S %	I %	R %	S %	I %	R %
Erythromycin(15 µg)	9(10.6%)	5(6.3%)	47 (82.9%)	0 (0%)	1 (100%)	0 (0%)
Clindamycin (2 µg)	30 (49.1%)	17 (27.5%)	14 (22.9%)	1 (100%)	0 (0%)	0 (0%)
Cefoxitin (30 µg)	48 (78.6%)	0 (0%)	13 (21.3%)			
Trimethoprim Sulpamethoxazole (25 µg)	37 (60.6%)	12 (19.6%)	12 (19.6%)			
Penicillin (10 µg)	0 (0%)	0 (0%)	61 (100%)	0 (0%)	0 (0%)	1 (100%)
Vancomycin (30 µg)	31 (50.8%)	27 (44.2%)	3 (4.9%)	5(62.5%)	3(37.8%)	0(0%)
Tetracycline(30 µg)	0 (0%)	7 (6.3%)	54 (93.6%)			
Gentamicin(10 µg)	10 (16.3%)	5 (8.1%)	46 (75.4%)			
Chloramphenical (30 µg)	2 (3.2%)	4 (6.5%)	55 (90.1%)			
Ciprofloxacin(5 µg)	30 (49.1%)	28 (45.9%)	3 (4.9%)			
Ceftriaxon(30 µg)				1(100%)	0(0%)	0(0%)

6.6 Antimicrobial susceptibility pattern in both hospital

A total of 4 different gram negative species of bacteria and two gram positive bacteria were isolated and identified, in which Patients the presence of bacterial had associated with wound infection. The overall antimicrobial susceptibility of penicillin show high resistance 39(84.7%)in Y12HMC and 12(54.5%) in AABET followed by clindamycin 4(86.9%) in Y12HMC ,.High level of sensitivity is shown by Amikacin, cefoxitin ,Tobramycin 98(91.7%),41(91.1%),18(78.2%),respectively in Y12HMC and also in AABET Amikacin ,Tobramycin, cefoxitin , 8(100%),10(90.9%),18(78.5%),followed by Ceftazidime 5(80%)

Table-9: Antimicrobial susceptibility pattern in both hospitals

Type of antibiotic	Antibiotic susceptibility pattern in Y12HMC			Antibiotic susceptibility pattern in AABET		
	S %	I %	R %	S %	I %	R %
Erythromycin(15 µg)	7(13.7%)	5(9.8%)	39(76.4%)	5(27.7%)	5(27.7%)	8(44.4%)
Clindamycin(2µg)	28(52.8%)	13(24.8%)	12(22.6%)	12(63.3%)	5(29.4%)	12(10.5%)
Cefoxitin(30 µg)	33(70.2%)	----------	14(29.7%)	4 (26.6%)	----------	11(73.3%)
Trimethoprim Sulpamethoxazole(25 µg)	30 (55%)	12 (20%)	7 (25%)	9(25%)	27(75%)	0(0%)
Vancomycin(30 µg)	24(47%)	24(47%)	3 (6.3%)	12(66.6%)	6(33.3%)	0 (0%)
Penicillin(10 µg)	0 (0 %)	2 (4.3%)	44 (95.6%)	0(0%)	3 (20 %)	12 (80 %)
Tetracycline(30 µg)	0 (0%)	3(6.3%)	44 (93.6%)	0 0(%)	4(22.2%)	10(71.4%)
Gentamicin(10 µg)	19(26.3%)	9 (12.5%)	44(61.1%)	10 (40%)	5 (20%)	10 (40%)
Chloramphenical(30 µg)	3 (5.5%)	5 (9.2%)	46 (85.1%)	2 (10%)	3(15%)	15 (75%)
Tobramycin(10 µg)	15(71.4%)	6 (28.5%)	0 (0%)	6 (60%)	3 (30%)	1(10%)
Ciprofloxacin(5 µg)	27(37.5%)	15 (20.8%)	30 (41.6%)	10 (40%)	9 (36%)	6(24%)
Ceftazidime(30 µg)	9 (39.1%)	6(26%)	12 (52.1%)	8 (80%)	0 (0%)	2 (20%)
Amikacin(30 µg)	21(80%)	5 (20%)	0 (0%)	9 (81.1%)	2(18.8%)	0 (0%)
Meropenam(10 µg)	21(84%)	4 (16%)	0 (0%)	5 (50%)	4 (40%)	1 (10%)
Cefepime(30 µg)	10(40%)	7 (28%)	8 (32%)	4 (36.3%)	4 (36.3%)	3 (27.2%)
Cefotaxime(30 µg)	8 (57.1%)	4 (28.5%)	2 (14.2%)	3 (60%)	2 (40%)	0 (0%)
Ampicillin(10 µg)	0(0%)	2 (25%)	6 (75%)	0 (0%)	1 (16.6%)	4 (83.3%)

7. Discussion

During our study, we observe a high bed occupancy rate in both hospitals. Patients are at a high risk of infection as a result of the nature of the burn injury itself, the immune-compromising effects of burns, prolonged hospital stays, and intensive diagnostic and therapeutic procedures. The coagulase-negative staphylococci (CoNS) are among the most frequently isolated bacteria in this study. These bacteria are normal inhabitants of human skin and mucous membranes and, therefore, one of the major challenges of daily diagnostic work is to distinguish clinically significant CoNS from contaminant strains (33). However it needs another study. Bacteria such as *S. aureus* and *P. aeruginosa* produce very critical virulence features, accountable for continuing infection and interruption remedial in chronic wounds. *S. aureus* causes clinically pertinent infections mostly because of its virulence factors such as coagulase, catalase, clumping-factor A and leucocidines. Similarly, the production of an elastase by *P. aeruginosa* has been linked to its pathogenicity in the wound situation. Thus, our outcomes settle the usual most prevalent microorganisms found in infected wounds (7). However, the role that each specific pathogen plays in both no healing and infected chronic wounds is not yet very defined. Beyond the presence of pathogens, it has been well-thought-out to be of supreme importance the presence of specific bacterial combinations and interactions in both acute and chronic wounds. The resistance to cefoxitin is particularly important because it can give us the percentage of methicillin-resistant *Staphylococcus aureus* (MRSA); in our study, a relevant percentage (40.3%) of *S. aureus* was cefoxitin resistant. *S. aureus* has always been a major source of infection in acute soft tissue wounds, but MRSA has only been an infecting organism in a small fraction of the total. Nevertheless, MRSA is flattering a more common wound pathogen. The occurrence of MRSA presents two problems: the first is associated to the chronic wound being a source of other MRSA nosocomial infections and the second is related to the impact of MRSA on the chronic wound itself, that is, who have chronic wounds growing MRSA and have an increased risk of suffering a bacteremia by MRSA (2).

In Y12HMC *CONS* was the predominant bacteria followed by *S.aureus* the result were similar from AABET which both bacteria were dominant. Other bacteria isolate in both hospitals were *P.aeruginosa*, *Acinetobacter* species, *Klebsiella* species, *Proteus species.* The number of bacteria species higher in Y12HMC except for *Proteus* species that found four times in both hospitals. The only bacteria isolate from Yekatit 12 hospital medical college was *Beta hemolytic streptococcus*. The total percentage of bacterial growth is 68% from Y12HMC and 90 % AABET.

On the other hand there is fungal growth from Y12HMC 21 yeast and 11 molds were isolated and from AABET both fungal growths are 17 times. From this growth of yeast *C.tropicalis* and *C.albican* are predominant in Y12HMC both are isolate eight times followed by *C.krusei* which is found five times. The

predominant yeast from AABET was *C.tropicalis* which is isolate 10 times and followed by *C.albican* which is isolate 5 times. When we see mold growth in both hospitals the predominate was *Penicillium* species which is found five times in AABET hospital most of the fungal growth counted one time except *Aspergillus* species and *Fusarium* species which is found twice from different sample.

In Antibiotic susceptibility Pattern there are multi-drug resistance bacteria found in both hospitals. *Klebsiella* species are the leading bacteria that show high resistance for most drugs in both hospitals and the opposite result found in *Acinetobacter* species shows a high sensitivity rate in most drugs. The most effective drug was Meropenem and Amikacin there were 84% and 80% effective respectively in both hospitals. Penicillin was 95% resistance in Y12HMC and 80% resistant in AABET and also Tetracycline, Chloramphenicol show greater than 70% resistance in both hospitals. Some drugs show different results in both hospitals. Erythromycin shows 76% resistance in Y12HMC and 44% resistance, Cefoxitin 70% sensitivities in Y12HMC, and 73% resistance AABET. These variations may be due to the amount of sample size differ and also variation from one bacterial gene to other.

In the present study, about 73.7% of the patients were in the ≥24year's age group and also 33.7% was less than three years. Males (51.7%) were the most affected compared to females (48.3%). Etiology of burns, due to scalds (47.1%) was the predominant cause among patients in our study followed by flame (39.6%) different results were recorded in other studies by Aisyah S. et al. (34) 76% were caused by flame and 19% were caused by electricity.

There is some research (data) in bacterial profile and drug susceptibility pattern of bacteria in burn patient in our county and it is limited but there is no single study regarding fungal pathogen however data presented in this study could provide information. The overall prevalence of bacterial infection of the present study was 64.1%.this prevalence was higher than the study conducted at felege hiwot referral hospital, North West Ethiopia (53%) Yekatit12 hospital, Addis Ababa (42%), and Imam Musa Kazem burn hospital in Isfahan, Iran (63.8%) (35, 15, 17).

This study is lower than the prevalence of wound infection reported from Jimma University Specialized Hospital, South-West Ethiopia (87.3%), University of Gondar Referral Hospital, Northwest Ethiopia (83.3.9%), Yekatit 12 hospital burn unit, Addis Ababa, Ethiopia (83.3%), Tertiary Care Center,India (89.5%), and the Burn Unit of the Hospital Regional da Asa Norte, Brasília, Brazil, (86.6%) (29, 14, 15, 15, 37).

The result of this study is similar to the report of Tertiary Care Center India (21) which showed that (22.9%) *Staphylococci aureus* isolate. Also the result of this study correlates with the report of N.P singh et al. (38) which showed that *Pseudomonas spp* and *Staphylococcus aureus* were the most prevalent bacteria isolated. It is also quite similar to the result of C. Manikandan and A. Amsath (40) which reported that *Klebsiella spp*

was the less prevalent bacteria isolated. This finding is also consistent with the study conducted by N. Agnihotri et al in *Proteus spp* findi the study was conducted as follows ng (14) other studies in Malawi and Nigeria also demonstrate similar finding on *S.auerus* was the predominate bacteria that isolate (41, 42). On the other hand, the result of this study is inconsistent with the work of Alireza Ekrami et al. (23) which showed that *Pseudomonas aeruginosa* was the predominant among the isolated bacteria. It also contradicted the work of yasemin bayram et al (5) which reported that *Acinetobacter* species was the most prevalent bacteria isolated. The result of this study clearly spelled out, couglase negative staphylococcus (22.9%), *Staphylococcus aureus* (22.5%) *Pseudomonas aeruginosa* (7.1%), *Acinetobacter* species (2.9%), Proteus species (3.3)*Klebsiella* species (2.1%), was the most prevalent bacteria isolated. *This result directly contradicted the report of yasemin bayram et al* (5) *which showed that Acinetobacter species (23.6%) was the most prevalent bacteria isolated, followed by Pseudomonas aeruginosa (12%), Staphylococcus aureus (11.2%) E. coli (10%),* Also, it is quite different from the work of Mohammed J. Alwan et al (24)which showed that *Pseudomonas aeruginosa* was found to be the most common isolate (48.9%) followed by *Staphylococcus aureus* (24.4%), *Citrobacter braakii* (13.3%), *Enterobacter spp*. (11.1%), Coagulase-negative Staphylococci (11.1%), *Proteus vulgaris* (6.66%), *Corynebacterium* spp. (6.66%), *Micrococcus* (6.66%), *Proteus mirabilis* (4.44%), *Enterococcus faecalis* (4.44%), *E.coli* (4.44%), *Klebsiella spp.* (2.22%), *Bacillus* spp. (2.22%), *Serratia macerscens* (2.22%) and *Serratia rubidia* (2.22%).

The difference in the frequency of the types of bacterial isolates between hospitals is most probably due to variation in maintaining standard personal hygiene, patients' population, cleaning producer, wound dressing and sometimes type of burn unit the attendant dressing procedure in each hospital. Gram-positive organisms that comprises of 110(54.8%) and 37(15.4) of the total isolate were found in both hospitals. Our study showed that, wounds harbor about 154(64.1%) of the Gram-negative and Gram positive bacterial isolates without the addition of mixed growth that count about 7(2.9 %).

On other side there was a Colonization of the burn wound with fungi is not a surprising phenomenon for other counties because they have information for us it is a surprise? In the present study, the overall isolation rate of fungus was found to be 27.5%. In the present study, we found that the most common isolates yeast were *Candida tropical* (17.5%), *Candida albican (11.2%) Candida krusai* (5.6%), *Aspergillus species* (7.8%), *pencillium species* (6.8%), *Mucor* (4.3), *Fusarium (*2%), *Alternari* (2.8%), other molds (3.2%) Other studies by James Ballard et al (57) Candida species (85%), yeast non-Candida (21%), *Aspergillus* (14%), other mold (39 9.0%), and others (1.4%) different to our study. In contrast, other studies by Rafik A et al. [49] an noted that The fungal pathogen most frequently isolated was Candida albicans (65.7%), followed by other Candida species (18.6%). Aspergillus spp was present in 3.9% and it differs from our finding. In the present study, predominate isolation rate of *Candida tropical* was high and similar

with the study of Sapana G. Mundhada (18).On the other hand, majority of the staphylococci aureus were multiple antibiotic resistant and these multi-drug resistance patterns had been documented already . In this finding, cefoxitin were found to be active against more than 78% of *S. aureus* isolates. A little similar with the work of Sapana G et al (15%) that showed 66% sensitivity of S.aureus to cefoxitin (38).In this study, 100% of the S. aureus of isolates were found to be resistant to Pencillin. Similar study in Northwestern Ethiopia gonder (30) On the other hand, in gram negative bacteria 100% Meropenam sensitivity seen in *Acintobacter* species this study quite different with 81.7% resistance reported both in Ethiopia and Elaham (17) , a. In this study, 75% Amikacin and Meropenan was reported as sensitivite to *P.aeruginosa* antimicrobial classes which is diiferent Ezzathlloh(43). *Klebsilla species* shows that sensitive for Amikacin this finding agrees with previous studies done India (21).

8. Strength and limitation of the study

8.1 Strength

This study has assessed the Bacterial and fungal infection and their drug susceptibility pattern in burn patients by using different microbiological culture media like Potato dextrose agar, Chrome agar, Blood agar, MaCconcy Agar, and Muller hint Agar and also biochemical test with drug susceptibility was done.

8.2 Limitation

- We did not do fungal drug susceptibility test.
- The study was limited to two government hospitals and their burn units.

9. Conclusion

The wounds were contaminated with various types of bacteria. This might be a reflection of the inappropriate use of antibiotics. The presence of MDR in the wound may be a factor for persisted infection. Adherence to infection prevention practices may be paramount important. Additionally, the government at all tiers should endeavor to sponsor researches on the development of new antibiotics that could be relevant in the treatment of severe infections caused by antibiotic-resistant bacteria.

Wound infections are still common health problems among burn patients in the study area, so the health professionals give attention not only to bacterial infections. They should give attention to fungal infection examinations and they should do one routine treatment of patients. Some of the participants in the study area had a positive fungal result. It is not contemptible it an alarming finding that needs more focus and follow-up on the fungal infection.

10. Recommendations

Based on the finding of the study the following three points were recommended.

I. There is need for hospitals to encourage periodic review of the microbial flora of their environment and the antibiotic sensitivity pattern.

II. Fungal examination should be routinely performed in the follow-up of patients.

III. It is also necessary that all professionals should take an active role in infection control within their organization and more resources should be provided to encourage good antibiotic Practice and good hygiene in hospitals.

11. REFERENCES

1. Kotz P, Fisher J, McCluskey P, Hartwell SD, Dharma H: Use of a new silver barrier dressing, allevyn Ag in exuding chronic wounds. Int Wound J 2009, 6:186–194

2. Dai T et,al Hamblin MR: Topical antimicrobials for burn wound infections. Recent Pat Antiinfect Drug Discov 2010, 5(2):124–151.

3. Siddiquee S. Practical handbook of the biology and molecular diversity of Trichoderma species from tropical regions. Cham: Springer International Publishing; 2017

4. Bayram Y, Parlak M, Aypak C, Bayram İ. Three-year review of bacteriological profile and antibiogram of burn wound isolates in Van, Turkey. International journal of medical sciences 2013; 10(1):19.

5. Bhat VG, Vasaikar SD. Bacteriological profile and antibiogram of aerobic burn wound isolates in Mthatha, Eastern Cape, South Africa. Southern African Journal of Epidemiology and Infection 2010; 25(4):16-9.

6. Poole MD. Are we facing the end of the antibiotic era? Ear, Nose & Throat Journal 1993; 72(6):433-.)

7. Dahal RH, Chaudhary DK. Microbial infections and antimicrobial resistance in Nepal: current trends and recommendations. The open microbiology journal 2018; 12:230

8. Struck MF, Gille J. Fungal infections in burns: a comprehensive review. Annals of burns and fire disasters 2013; 26(3):147

9. Agnihotri N, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms—a five-year study Burns 2004 ;30(3):241-3

10. Stokes MA, Johnson WD. Burns in the Third World: an unmet need. Annals of burns and fire disasters 2017; 30(4):243.

11. Naqvi SZ, Naqvi SA, Usman M, Naqvi SB. burn wound infection. The Professional Medical Journal 2014; 21(05):869-73

12. Struck MF, Gille J. Fungal infections in burns: a comprehensive review. Annals of burns and fire disasters 2013; 26(3):147.

13. Mohammed A, Seid ME, Gebrecherkos T, Tiruneh M, Moges F. Bacterial isolates and their antimicrobial susceptibility patterns of wound infections among inpatients and outpatients attending the University of Gondar Referral Hospital, Northwest Ethiopia. International Journal of Microbiology 2017.

14. Haghighifar E, KamaliDolatabadi R. Bacterial Infections and Antimicrobial Resistance Patterns of Burn Wound Infections: A One Year Study from Burn Hospital, Isfahan, Iran. Journal of Advances in Medical and Biomedical Research 2020; 28(128):144-50.

15. Alaghehbandan R, Azimi L, Lari AR. Nosocomial infections among burn patients in Teheran, Iran: a decade later. Annals of burns and fire disasters 2012; 25(1):3.
16. Rezaei E, Safari H, Naderinasab M, Aliakbarian H. Common pathogens in burn wound and changes in their drug sensitivity. Burns 2011; 37(5):805-7.
17. Kaushik R, Kumar S, Sharma R, Lal P. Bacteriology of burn wounds—the first three years in a new burn unit at the Medical College Chandigarh. Burns. 2001; 27(6):595-7.
18. Leela KV et.al Antibiotic sensitivity pattern of bacterial isolates in burns wound infection in a tertiary care hospital. IPP. 2016; 3:726-9.
19. Singh NP, Goyal R, Manchanda V, Das S, Kaur I, Talwar V. Changing trends in bacteriology of burns in the burns unit, Delhi, India. Burns. 2003; 29(2):129-32.
20. Manikandan C, Amsath A. Antibiotic susceptibility of bacterial strains isolated from wound infection patients in Pattukkottai, Tamilnadu, India. Int J Curr Microbiol App Sci. 2013; 2(6):195-203.
21. Mundhada SG, Waghmare PH, Rathod PG, Ingole KV. Bacterial and fungal profile of burn wound infections in tertiary care center. Indian Journal of Burns. 2015; 23(1):71
22. Song W, Lee KM, Kang HJ, Shin DH, Kim DK. Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. Burns. 2001; 27(2):136-9.
23. De Macedo JL, Santos JB. Nosocomial infections in a Brazilian burn unit. Burns. 2006; 32(4):477-81.
24. Macedo JL, Santos JB. Bacterial and fungal colonization of burn wounds. Memórias do Instituto Oswaldo Cruz. 2005 ;100(5):535-9.
25. Alebachew T, Yismaw G, Derabe A, Sisay Z. Staphylococcus aureus burn wound infection among patients attending Yekatit 12 hospital burn unit, Addis Ababa, Ethiopia. Ethiopian journal of health sciences. 2012; 22(3).

26. Mohamed H. One year prevalence of critically ill burn wound bacterial infections in surgical ICU in Egypt: Retrospective study. Egyptian Journal of Anaesthesia. 2016 1;32(3):431-4.

27. Shittu AO, Kolawole DO, Oyedepo EA. A study of wound infections in two health institutions in Ile-Ife, Nigeria. African journal of biomedical research. 2002; 5(3).
28. Mohammed A, Seid ME, Gebrecherkos T, Tiruneh M, Moges F. Bacterial isolates and their antimicrobial susceptibility patterns of wound infections among inpatients and outpatients attending the University of Gondar Referral Hospital, Northwest Ethiopia. International Journal of Microbiology. 2017.
29. Mama M, Abdissa A, Sewunet T. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia. Annals of clinical microbiology and antimicrobials. 2014; 13(1):14

30. Aisyah S, Yulia R, Saputro ID, Herawati F. Evaluation of antibiotic use and bacterial profile in Burn Unit Patients at the dr. Soetomo General Hospital. Annals of burns and fire disasters. 2018; 31(3):194

31. Alebachew T, Yismaw G, Derabe A, Sisay Z. Staphylococcus aureus burn wound infection among patients attending Yekatit 12 hospital burn unit, Addis Ababa, Ethiopia. Ethiopian journal of health sciences. 2012; 22(3).

32. Hashim SM. *Isolation and Identification of Mycelial Fungi Associated with Human Ear Infections* (Doctoral dissertation, University of Khartoum).

33. Kulkarni V, Arali SM, Jayaraj YM, Shivannavar CT, Joshi MR. Bacterial etiology and their antibiogram in burn wound infections at Kalaburgi region (India). Indian Journal of Burns. 2015; 23(1):65.

34. Aisyah S, Yulia R, Saputro ID, Herawati F. Evaluation of antibiotic use and bacterial profile in Burn Unit Patients at the dr. Soetomo General Hospital. Annals of burns and fire disasters. 2018; 31(3):194

35. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clinical microbiology reviews. 2006; 19(2):403-34

36. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clinical microbiology reviews. 2006; 19(2):403-34

37. Oncul O et al. The evaluation of nosocomial infection during 1-year-period in the burn unit of a training hospital in Istanbul, Turkey. Burns. 1;28(8):738-44

38. Qader AR, Muhamad JA. Nosocomial infection in Sulaimani burn hospital, Iraq. Annals of burns and fire disasters. 2010; 23(4):177.

39. Yildirim S, et al , Haberal M. Bacteriological profile and antibiotic resistance: comparison of findings in a burn intensive care unit, other intensive care units, and the hospital services unit of a single center. The Journal of burn care & rehabilitation. 2005; 26(6):488-92.

40. Taiwo SS, Okesina AB, Onile BA. Invitro antimicrobial susceptibility pattern of bacterial isolates from wound infections in University of Ilorin Teaching Hospital. African Journal of Clinical and Experimental Microbiology. 2002; 3(1):6-10.

41. Mosier MJ, Pham TN. American Burn Association Practice guidelines for prevention, diagnosis, and treatment of ventilator-associated pneumonia (VAP) in burn patients. Journal of Burn Care & Research. 2009; 30(6):910-28.

42. Yildirim S, et al , Haberal M. Bacteriological profile and antibiotic resistance: comparison of findings in a burn intensive care unit, other intensive care units, and the hospital services unit of a single center. The Journal of burn care & rehabilitation. 2005; 26(6):488-92.

43. Sengupta S, Kumar P, Ciraj AM, Shivananda PG. Acinetobacter baumannii—an emerging nosocomial pathogen in the burns unit Manipal, India. Burns. 2001; 27(2):140-4.
44. Godebo G, Kibru G, Tassew H. Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Annals of clinical microbiology and antimicrobials. 2013; 12(1):17.
45. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clinical microbiology reviews. 1996; 9(4):499-511.
46. Appelgren Pet al U. A prospective study of infections in burn patients. Burns. 2002; 28(1):39-46.
47. Sengupta S, Kumar P, Ciraj AM, Shivananda PG. Acinetobacter baumannii—an emerging nosocomial pathogen in the burns unit Manipal, India. Burns. 2001; 27(2):140-4.
49. Kulkarni V, Arali SM, Jayaraj YM, Shivannavar CT, Joshi MR. Bacterial etiology and their antibiogram in burn wound infections at Kalaburgi region (India). Indian Journal of Burns. 2015; 23(1):65.
50. Kaur H, Bhat J, Anvikar AR, Rao S, Gadge V. Bacterial profile of blood and burn wound infections in burn patients. Burns. 2006; 34:89-95.
51. Miller JM. Handbook of Specimen Collection and Handling in Microbiology: J. Michael Miller. US Department of Health and Human Services, Public Health Service, Centers for Disease Control; 1985.
52.Rafla K, Tredget EE. Infection control in the burn unit. Burns. 2011; 37(1):5-15.

Annexes

Annex I: English Versions of Participant Information Sheet

Title of the Research

Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Aabet Hospitals, Addis Ababa Ethiopia.

Principal investigator: Andualem Garedew (BSc)

Advisor: Adane Bitew (PHD)

Name of the Organization: School of Medical Laboratory Technology, Addis Ababa University

My name is Andualem Garedew and I am a student at Addis Ababa University for master`s degree .I am doing a research on Bacterial and fungal infection and their drug susceptibility pattern in burn patients as a part of my study course. If you are willing to participate in this research study you are requested to sign the consent form.

Introduction

Title of the Research is '' Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Abate Hospitals, Addis Ababa .'' I am doing a research on this title. This research will be voluntary based and will help to assess the Nosocomial infection in burn patient.

Expected from participants

What we expect from you is your willingness to give us wound swab. Sample will be collected using sterile cotton swap and test tubes by experienced nurse. Being asked to give sample does not necessarily 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. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required participating

You will spend 10-15 minutes until the specimen is collected and permission form is signed.

Potential risks and Discomforts

Specimen collection will have no effect and you will not get any risk as the sample will be collected by well trained professionals. The sampling procedure if cause any discomfort. But we will try to minimize the discomfort as much as possible. We offer you necessary medical treatment freely.

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 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 wound infection. You will also obtain all the results of the analysis for free 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. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are:
Investigator: Andualem Garedew (BSc), DMLS; AMU, +251913647800

Email- andu21andi@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU +251911039162

For additional information, please contact Addis Ababa University, College of Health Sciences, and Department of Medical Laboratory Sciences at: Telephone +251112755170

Annex II: Amharic Versions of Participant Information Sheet

እኔ አንዱአለም ጋረደው እባላለሁ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ስራየን በመስራት ላይ እገኛለሁ፡፡ በመሆኑም እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል በጥናት ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ፡፡

<u>ከጥናቱ ተሳታፊ የሚጠበቁ</u>

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙና እንዲወሰድ እና ለጥናቱ እንዲዉል መስማማት ይጠበቅቦታል፡፡ የጤና ባለሙያ ከእርሰዎ ናሙናዉን ይሰበስባል ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል፡፡ ይሁን እንጂ ይህ ዓይነቱ መረጃ የእርሶን ማንነት የሚገልጹ ማስረጃዎን ማለትም ስም አድራሻና የመሳሰሉት መረጃዎች አይጨምርም፡፡ ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ ናሙና ሰጡ ማለት በሽታው ይገኝብዎታል ማለት አይደለም በእርሰዎ ናሙና ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈላጊውን ህክምና ያገኛሉ፡፡

<u>ተሳታፊዉ የሚያጠፋዉ ጊዜ</u>

የተዘጋጀዉን የስምምነት ቅጽ ለመፈረምና ናሙና ለመስጠት 10-15 ደቂቃ ያስፈልጋል፡፡

<u>በጥናቱ በመሳተፍ የሚስከትላቸዉ ችግሮች</u>

ናሙና በሚሰበሰብበት ወቅት ምነም አይነት ችግር አያስከትልቦትም፡፡ ሆኖም ናሙናዉ በሚወሰድበት ጊዜ ትንሽ የህመም ስሜት ሊኖር ይችላል፡፡ የተለየ የሕመም ስሜት ከተሰማዎት አስፈላጊዉን እርዳታ በነፃ እናደርጋለን፡፡

<u>የመረጃዉ ሚስጥራዊነት</u>

ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦረቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፤ ይህን ሚስጥር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ፡፡ ከዚህም በላይ ስለ እርስዎ ያለዉን ማንኛዉንም መረጃ የይለፍ ቃል ባለዉ የኮፒዉተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል፡፡

<u>በጥናቱ በመሳተፍ የሚያስከትላቸዉ ጥቅሞች</u>

ይህ ጥናት የማስተርስ ዲግር መመረቂያ እንደመሆኑ መጠን በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማ ጥቅም የለም፡፡ለወደፊት በተመሳሳይ ሁኔታ ዉስጥ ላሉ በሽተኞች በመረጃ ላይ የተመረተ ህክምና ለመስጠት ያግዛል፡፡ ከፈለጉ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ እንዲሁም ስለ አስፈላጊዉ ህክምና ከሀኪምዎ ጋር ይነጋገራሉ፡፡

<u>የጥናቱ ተሳታፊዎች መብት</u>

ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተና ተሳትፎዎን መተውና በማንኛውም ሰዓት ጥናቱን ማቆም ይችላሉ፡፡ በጥናቱ ዉስጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማቋረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም፡፡ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠቅና ገለፃ የማግኘት መብት አለዎት፡፡የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል፡፡

<u>ግንኙነትና ጥያቄ</u>

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዉ ድንገተኛ ችግር ወይም ጥያቄ ካሎት በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ተመራማሪ፡- አንዱአለም ጋረደው (ቢ.ኤስ.ሲ) ሞባይል +251913647800

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይል፤ andu21andi@gmail.com

አማካሪ ዶ/ር አዳነ ቢተዉ (ፒ.ኤች. ዲ) ሞባይል +251911039162

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ

ስልክ-+251112755170

ከዚህ በታች የሚገኘው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን መስማትዎን እና መገንዘብዎን የሚያሳይ ነው፡፡ ከመፈረምዎ በፊት እባክዎትን የጥናቱን ዓላማ የተሳትፎ ጉዳትና ጥቅሙ የመተው የማቋረጥ መብትና ነፃነት እንዳለዎት ይረዱ፤ተስማምተዋል? ጥናቱን መግለጫ አንብብያለሁ/ ሰምቻለሁ እናም ተረድቻለሁ መመሪያውን እንደሆነና በእኔም ሊከሰት እንደሚችል ተረድቻለሁ፡፡ በጥናቱ ላይ ለመሳተፍተስማምቻለው

እኔ_____ፊርማ_____ቀን_____

Annex III: English Versions of Consent form

Card no…..……

I had been informed that the objective of this study is Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Abate Hospitals, Addis Ababa. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of burn patient in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that wound swab sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Please direct any questions or problems you may encounter during this study to:

Andualem Garedew
Department of Medical Laboratory Sciences, College of Health Sciences
Addis Ababa University
Mobile +251913647800
Email- andu21andi@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences,
Department of Medical Laboratory Sciences at: Telephone +251112755170

Annex IV: **Amharic Versions of Consent form**

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር _____

የተሳታፊዉ ስም_____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Abate Hospitals, Addis Ababa Ethiopia” **ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፤ለጥናቱም የቁስሉ መግል ፈሳሽ** (wound swab) **ናሙና እንደሚያስፈልግ ተገልጾልኛል፤የጥናቱንም አላማዎችም ተረድቻለሁ፡፡በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቱ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡**

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም _____**ፊርማ**_____**ቀን**_____

ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ፡፡

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የጤና ሳይንስ ኮሌጅ አዲስ አበባ ዩኒቨርሲቲ ኢ-ሜይል andu21andi@gmail.com

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ የሕክምና ላብራቶሪ ሳይንስት ክፍል ይጠይቁ ስልክ+251112755170

Annex V: Assent Form for Children Participant (English Version)

Name of study participant ----------

Name of the participant's family or Guardian ----------

I _____ understand that my parents /guardian have/has given permission for me to take part in a research entitled as ""Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Aabate Hospitals, Addis Ababa Ethiopia"

The study participant mentioned above who is not able to give informed consent himself because

He/she is younger than 18 years not allowed to decide on him/herself.

- I agree samples to be collected from me.
- I understand that the information will be confidential.
- I understand that I can stop the study at any time.
- I understand it will not affect my current and future medical services.
- I agree that there is no payment as compensations for me.

It is therefore with full understanding; by taking a full responsibility, I gave my assent voluntarily to the researcher to use the information and specimen for this study.

Participant code: _____

Participant's signature/Finger print ---------- Date: ----------/--------/----------

Participant's family/Guardian signature ---------- Date: ----------/--------/----------

Annex VI: Assent form for Children Participant (Amharic Version)

የተሳታፊዉ ልጅ ስም …….

የተሳታፊዉ ልጅ ወለጅ ወይም አሳዳጊ ስም ……..

እኔ----------የተባልኩ ልጅ ወላጆቼ /አሳዳጊዎቼ

“Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Abate Hospitals, Addis Ababa Ethiopia”

በሚል ርዕስ ለሚካሄደዉ ጥናት እንድሳተፍ መስማማታቸውን ተረድቻለሁ፡፡

- **እኔም ናሙና ለመስጠት ተስማምቻለሁ፡፡**
- **ከእኔ የሚስበስበዉ መረጃዎች በሚስጥር እንደሚያዙ ተረድቻለሁ፡፡**
- **በማንኛዉም ሰዓት ከጥናቱ ማቋረጥ እንደምችል ተረደቻለሁ፡፡**
- **በጥናቱ ባለመሳተፌ/በማቋረጤ አሁን/ወደፊት የህክምና አግልጋሎቴ ላይ ችግር እንደማይፈጥርብኝ ተረደቻለሁ፡፡**
- **እኔ በጥናቱ በመሳተፌ የማገኘዉ የገንዘብ ክፊያ እነደሌለ ተረደቻለሁ፡፡**

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባ ተረድቼ የሚያስፈለገዉ ናሙና እና በቂ መራጃ ለላቦራቶሪ ምርማረ ለመስጣት ተስማሚቼላዉ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የተሰተፊዉ መለያ ቁጥር: _____ ቀን: ----------/--------/----------

የተሳታፊዉ ልጅ ወለጅ ወይም አሳዳጊ ፊርማ---------- ቀን: ----------/--------/----------

Annex VII: Data Collection Form

For The Study "Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12 and Aabet Hospitals, Addis Ababa Ethiopia. "

Questioner prepared by English language

Interviewer language English, Amharic, Afan Oromo, Tigrigna, Other _____

Study ID…….. Study Serial Number…….

1.0 Participant Socio-demographic Data

Date of data collection: ……

Patient Code Number: ……. Data Collector's initials: …….

1. Age: ……Years

2. Sex M [ ] F [ ]

3. Place of residence: A. Urban B. Rural

4. Highest level of Education A. Informal B. Primary C. Secondary D. College

5. Marital Status A. Married B. Single C. Divorced D. Widowed E. Separated

6. Employment Status A. Employed B. Not Employed C. Student D. Retired

II. Association of clinical profiles of respondents with wound infection.

1. A) Etiology/cause of burn: Scalds: [ ] flame [ ] chemical [ ] Electrical [ ] others [ ]

2. Anatomical site affected- Head [] Neck [] Trunk [] head [] extremities [] whole body part [] other----?

3. Level of burn wound A) I Degree B) 2ND Degree C)3rd degree D)4th degree?

4. Has any part of the wound leaked clear thick and yellow /green fluid/ pus /purulent/?

5. Total body surface area/TBSA----------?

7. Have you been given antibiotic for a problem with your wound? □Yes □ No

8. Date of Admission?

9. Others (Specify)……

OUTCOME OF LABORATORY INVESTIGATION

1- Name of pathogen isolated……
2- Antibiotic that are sensitive against the isolate……
3- Antibiotic that are intermediate against the isolate……..
4- Antibiotic that are resistance against the isolate……..

Contact person –Name ……phone no……

Name and signature of the data collector/interviewer……..

THANK YOU FOR PARTICIPATION AND COOPERATION.

Annex VIII: Laboratory test Procedure for wound swab Specimen Collection and Processing

A. **Sample collection:** For diagnosis of the pathogen two wound swap will collect from the study subjects by experienced nurse

Sample collection procedure

1. Cleanse the wound margins and superficial area thoroughly with sterile saline
2. Use cotton or synthetic –tipped swab to obtain a sample.
3. Changing sponges with each application. Remove all superficial exudates. Remove overlying debris with a scalpel and swabs or sponges.
4. Collect a sample from the base or advancing margin of the lesion. Collect sufficient tissue samples, avoiding necrotic areas.
5. Place tissue in a sterile container with a small amount of non-bacteriostatic saline (just enough to keep the specimen from drying out).

B. **Culture for Bacteria**

1. The wound swab will be inoculated onto BAP,Mac,SDA,MSA.
2. All culture plates will be inoculated at 37^{o}C for 24 hours.
3. Bring the culture Medias to Room Temperature.
4. Make a single line down the middle of the plate by rolling the swab in to the culture media. Cross-streak the line of material with a series of very close streak lines such that the entire plate surface is utilized
5. Incubate plates 16 - 24 hours at 35 – 37^{o}C aerobically
6. If there is growth after 16-24 hours of incubation, Perform definitive biochemical identification and sensitivity test

C. **Identification of Bacteria**

Catalase

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Principle

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

Procedure

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2. 2 Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution. Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.
3. Look for immediate bubbling
4. Results Active bubbling . . . . . Positive catalase test

   No bubbles . . . . . Negative catalase test

Coagulase

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

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus*: Free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test. Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulasereacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test. A tube test must always be performed when the result of a slide test is not clear, or when the slide test is negative and Staphylococcus has been isolated from a serious infection.

DNA-ase test

This test is used to help in the identification of *S. aureus* which produces deoxyribonuclease (DNAase) enzymes. The DNA-ase test is particularly useful when plasma is not available to perform a coagulase test or when the results of a coagulase test are difficult to interpret.

Principle

Deoxyribonuclease hydrolyzes deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNA-ase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNA-ase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis

Method

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

2 Using a sterile loop or swab, spot-inoculate the test and control organisms. Make sure each test area is labelled clearly.

3 Incubate the plate at 35–37 C overnight.

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

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

Results Clealing around the colonies . . . . . DNA-ase positive strain

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

Oxidase

The oxidase test is used to assist in the identification of *Pseudomonas, Neisseria, Vibrio, Brucella,* and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase.

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used (see later text). When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

D. **Culture for fungus**

1. The wound swab will be inoculated onto Sabouroud (Potato) Dextrose agar supplemented with antibacterial antibiotic.

2. All culture plates will be inoculated at 30 - 34^{o}C for at least 3-4 week.

A. **Sample collection:** For diagnosis of fungal pathogen one wound swab will collect from the study subjects by nurse.

Sample collection procedure

1. Wash the wound in normal saline.

2. Use cotton tipped swab to obtain a sample of wound swab.

B. **Culture for yeasts**

1. The wound swab will be inoculated onto Sabouroud (Potato) Dextrose agar supplemented with antibacterial antibiotic.

2. All culture plates will be inoculated at 30 - 34^{o}C for 3 – 4 week.

3. Yeasts will be identified by inoculating CHROMagar.

C. **Identification of yeast**

BY the color they produce in CHROM agar we identify one yeast from other.

Interpretation

- C.albican - Green
- C.tropicalis - Blue
- C.krusei - Pink

Annex IX:

Declaration

Title of project: "Bacterial and fungal infection and their drug susceptibility pattern in burn patients admitted to Yekatit 12HMC and Aabet , Addis Ababa."

I, the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

Principal investigator:

Name: Andualem Garedew (BSc, MSC Candidate)

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____07/11/2021_____

Advisor (s):

Name: Adane Bitew (MSc, PhD)

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

Advisor (s):

Name: Zerihun W/Senbet (BSC, MSc)

Address:-Yekatit 12Hospital Medical College Laboratory Sciences

Signature: _____ Date: _____07/11/2021_____