ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

BACTERIAL PROFILE AND DRUG RESISTANCE PATTERN OF PATHOGENS ISOLATED FROM WOUND INFECTION AT ARMED FORCE REFERRAL AND TEACHING HOSPITAL, ADDIS ABABA, ETHIOPIA

BY: SOSINA AYALEW (BSc.)

ADVISOR: GEBRU MULUGETA (MSc.)

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY SPECIALTY)

June , 2014

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Bacterial profile and drug resistance pattern of pathogens isolated from wound infection at Armed Force Referral and Teaching Hospital, Addis Ababa, Ethiopia.

By

Sosina Ayalew (BSc.)

Approved by the Examining Board

_____	_____
Chairman, Dep. Graduate Committee	Signature
_____	_____
Advisor	Signature
_____	_____
Examiner	Signature

Acknowledgment

First of all I would like to thank the almighty God who gave me the courage and power to finish this paper. My Sincere thanks and appreciations are forwarded to my advisor Mr. Gebru Mulugeta for his support and professional advice for the successful completion of this research thesis. Also am great full for Department of Medical Laboratory Science for giving me an opportunity to conduct this research.

I would like to express my heartfelt gratitude to Armed Force Referral and Teaching Hospital for allowing me to do my project in the hospital and for their full support

My special thanks also go to all nurse staffs who supported me by identifying the target patient with careful clinical examination and sample collection.

My very great gratitude also goes to all laboratory professional of the hospital for their unreserved support throughout the project.

Table of content

List of abbreviation

ARFTH	Armed Force Referral and Teaching Hospital
BAP	Blood Agar Plates
CLSI	Clinical and Laboratory Standards Institute
EHNRI	Ethiopian Health and Nutrition Research Institute
EMLA	Ethiopian Medical Laboratory Association
KIA	Kligler iron agar
MDR	Multi-Drug Resistant
MRSA	Methicillin Resistant *S. aureus*
NIs	Nosocomial Infections
SOP	Standard Operating Procedures
SPP	species
SPSS	Statistical Package for Social Sciences
SSI	Surgical Site Infection

List of Tables

Title of the table **Page No.**

Summery

Introduction: - Wound infections are associated with increased morbidity and mortality. Etiologic agents of wound infections vary with geographical locations. Pathogens that infect wounds can be part of normal flora or acquired from the hospital environment.

Objectives: The aim of this study was to investigate the profile of pathogens cultured from infected wound and determine their antimicrobial resistance pattern to commonly prescribed antibiotics.

Methods: prospective cross sectional study was conducted at ARFTH from December 2013 to May 2014. Swabs from different types of wounds were processed to investigate etiologic agents using standard microbiological technique. Antimicrobial susceptibility tests were done using disc diffusion technique as per the standard modified Kirby-Bauer method.

Result

Out of 300 wound swab samples analyzed, 205(68.3%) were culture positive. 33 (16.1%) of the culture had double infections. and total 238 bacteria were isolated from 205 cases.. *Staphylococcus aureus* was the most frequently isolated pathogen which accounted for 91 (38.2%) of isolates followed by *Pseudomonas aeroginesa* 53 (22.3%). The sensitivity rates of norfloxacin, gentamicin and ceftriaxone were 82.8%, 78.9%, and 76.9% respectively. The overall MDR (resistant to three or more antibiotics) rate of gram positive bacteria were 73.6% and 67.6% of the gram negative bacterial isolates were identified as multiple drug resistants.

Conclusion

S. aureus and *psuedomonas aeroginesa* were the predominant causes of wound infections. norfloxacin Gentamicin and ceftraxone were the most effective drugs. Periodic surveillance of the species of bacteria involved in wound infection and determination of their antimicrobial resistance is recommended for empirical treatment.

Keyword wound infection, bacterial profile, drug resistance pattern

1. Introduction

Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity.[1] The exposed subcutaneous tissues provides a favorable substratum for a wide variety of microorganisms to contaminate and colonize, and if the involved tissue is devitalized and the host immune response is compromised, the conditions become optimal for microbial growth. This is because the host immune response plays a critical role in determining whether wound infection will arise.[2] Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues.[3-4] Wounds can also broadly categorized as having either an acute or a chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries. In marked contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue .[5]

All wounds are contaminated by both pathogens and body commensals. But The progression of a wound to an infected state is likely to involve a multitude of microbial or host factors .These may be the microbial load, and the combined virulence expressed by the types of microorganisms involved, patient risk factors like the general health and immune status of the host ,diabetes, cigarette smoking, obesity, and coincident remote site infections or colonization and operation-related risk factors including prolonged hospital stay before surgery, duration of the operation, tissue trauma, poor hemostasis, and foreign material in the wound, with these last greatly increasing the risk of serious infection despite a relatively small bacterial inoculums.[2,6-8]

Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites. Both acute and chronic wounds are susceptible to contamination and colonization by a wide variety of aerobic and anaerobic microorganisms.[5] Isolates that have been incriminated in cases of wound infections include: *Staphylococcus aureus*, *Staphylococcus epidermidis* , *Streptococcus faecalis, Streptococcus pyogenes ,Proteus mirabilis, Pseudomonas aeruginosa,*

Klebsiella spp, Escherichia coli, , Acinetobacter, and Enterobacter .Candida albicans and C. tropicalis have also been implicated as etiological agents.[3,9]

The majority of wounds are characterized by a polymicrobial aerobic-anaerobic microflora; therefore, the careful use of broad spectrum antimicrobial agents is likely to be the most successful treatment in the management of infected wound. However, various antibiotics are frequently and sometimes inappropriately prescribed or administered in wound treatments, which often leads to the selection of antibiotic-resistant bacteria strains.[10] Antimicrobial resistance among pathogens of wound infections is on the increase.[7] Antimicrobial drug resistance can be acquired as a result of mutation or acquisition of resistance genes via horizontal gene transfer, or can be an innate feature of an organism that is encoded chromosomally.[11] Antimicrobial drugs overuse, over dosing, drugs prescription with improper susceptibility test, self-medication and long duration of hospitalization was suggested to augment the problem of multi-drug resistant (MDR) in developing nations. [12]

2. Statement of the problem

Since wound colonization is most frequently polymicrobial involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected. In the event of infection, a wound fails to heal, the patient suffers increased trauma, treatment costs rise, and general wound management practices become more resource demanding.[5] Infection continues to be a major complication of wounds with significant increase morbidity and potential mortality.[3] Wound infection is one of the most challenging aspects of wound management and a major contributor to healthcare costs globally.[13] Wound infections may occur following accidental trauma and injections, but post-operative wound infections in hospital are most common.[3]

Wound infections are the most expensive complications following surgery and moreover, it is thought to be second most common type of nosocomial infections [14] Nosocomial infections (NIs) are the infections acquired during hospital stay and are widespread. They are important contributors to morbidity and mortality. These infections concern 2 million cases annually worldwide i.e., 5-15 per cent of hospitalized patients and up to 10 per cent of patients acquire more than one of these infections.[15]

The rate of surgical site infection (SSI) varies greatly worldwide and from hospital to hospital. In European hospitals, the overall rates of SSI range between 3% and 4% of patients undergoing surgery. Depending on the nature of surgery in question, the incidence of SSI ranges between <1% to >10%.[16]Epidemiological Study of Surgical Wound Infections conducted in India reported that the annual incidence of SSI to be 30.2 patients per 1000 patients.[15] It has been estimated that that 500,000 SSIs occur annually in the United States[17]and account for approximately one quarter of the estimated 2 million NI in the United States.[18] In Africa the rate of SSIs varied from 2.5% to 30.9% following various types of surgical procedures.[19] Studies have shown that the average hospital stays doubled and that the cost of hospitalization was correspondingly increased when postoperative surgical wound infection developed .[18] A study from Ethiopia reported that the mean postoperative stay and mortality were significantly higher in patients with surgical site infection compared with in uninfected patients.[20]

One to two percent of the population in the developed countries will experience a chronic wound in their lifetime.[21] in united state chronic wounds affect around 6.5 million patients. It is claimed that an excess of US$25 billion is spent annually on treatment of chronic wounds and It is expected that the number of chronic wounds will increase worldwide due to the increase of lifestyle diseases, such as diabetes, obesity, and cardiovascular diseases.[16]

Disability and loss of wages related to chronic wounds represents a heavy socioeconomic burden.[22]For many patients wounds are a significant and preventable barrier to the successful recovery or management of, a wide range of medical conditions. Wounds cause pain, suffering, sepsis, infection, nausea, fatigue, depression, psychological disturbances, loss of function, loss of mobility and personal financial cost. [23].

The rapid emergence of antimicrobial resistance among bacteria is a public health crisis. Wound Infections with antimicrobial-resistant bacteria increase patient morbidity and mortality and greatly increase the cost of medical care. [24] The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by methicillin resistant *S. aureus* (MRSA) and polymicrobic flora.[7]

There is now a well-recognized increase in MRSA colonization and infection in chronic wounds. The increase appears to be comparable to the worldwide increase in MRSA in acute wounds. The MRSA presents two problems, the first relates to the chronic wound being a source of other MRSA NI and the second relates to the impact of MRSA on the chronic wound itself. [25]

In most developing countries like Ethiopia, it is a common practice that antibiotics can be purchased without prescription. This leads to misuse of antibiotics by the public thus contributing to the emergence and spread of antimicrobial resistance.[26] The current spread of MDR bacteria pathogens has added a new dimension to the problem of wound infections. A regular bacteriological review of infected wounds is therefore a necessity if affected patients must receive quality health care, particularly when blind treatment is a necessity, as in underdeveloped and developing nations.[3]

3. Significance of the study

Bacteriological studies have shown that a wound infection is universal and that the types of bacteria vary with geographical locations.[9] The prevalence of different bacteria in infected wounds varies and the knowledge of prevalence in an institution cannot be extrapolated to others. Apart from inter-institutional variation, trans-institutional variation also exists.[26] However, studies assessing the etiological agents of wound infections in Ethiopia are very scarce.[27] Knowledge of the causative agents of wound infection and the extent of drug resistance of these isolates against different antimicrobial classes in a specific geographic region will therefore be useful in order to provide locally applicable data and to guide empirical therapy. Accordingly, the study finding is important in setups where immediate culture and sensitivity tests are difficult, sound epidemiological knowledge of bacterial pathogens helps in rationale selection of antibiotics for empiric treatment options. Thus, morbidity and mortality associated with wound infections by bacteria provide a strong argument for our intention to identify possible bacterial pathogens from patients hereby implementing strict rules to control their spread.

4. Literature review

A study was carried out to determine antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in Nepal. Out of 120 pus swabs processed for culture *Staphylococcus aureus* 36 (37.5%) was the predominant gram positive isolate and *Escherichia coli* 24 (25%) was the major gram negative isolate. All *S. aureus* isolates were sensitive to aminoglycosides and vancomycin. Out of 36 *S. aureus*, 15 (41.66%) isolates were methicillin resistant *S. aureus* (MRSA). *Staphylococcus epidermidis* showed high resistance (50% - 100%) to all antibiotics but were sensitive to vancomycin. All gram negative isolates showed high resistance against cephalexin (75% - 100%) and ceftriaxone (25% - 100%). Overall multi-drug resistant isolates were 66.7%. [3]

Another survey was conducted in Italy to assess Epidemiology and Microbiology of Surgical Wound Infections. This study included 676 surgery patients with signs and symptoms indicative of wound infections. Bacterial pathogens were isolated from 614 individuals. Among the common pathogens were *Staphylococcus aureus* (191 patients, 28.2%), *Pseudomonas aeruginosa* (170 patients, 25.2%), *Escherichia coli* (53 patients, 7.8%), *Staphylococcus epidermidis* (48 patients, 7.1%), and *Enterococcus faecalis* (38 patients, 5.6%). [28]

A prospective study was carried out in Nigeria to determine Microbiology of Wound Infections and its Associated Risk Factors. The overall prevalence of wound infections was 64.8%. The prevalence of wound infections was not significantly affected by gender but was significantly affected by age .The prevalence of wound infections was minimum among age group of <5 years old (20.0%) and maximum among the age group of 36-40 years old (77.5%). *Staphylococcus aureus* was the most prevalent etiologic agent (21.5%). β-lactams, fluoroquinolones and gentamicin were the most effective antibacterial agents. [9]

Similar study was done in Cameron to determine the bacterial profile of surgical site infection. Out of 110 (9.2%) patients who developed SSI, the isolated bacteria were Enterobacteriaceae (41.2%), *Staphylococcus aureus* (15.3%), *Pseudomonas spp.* (14.1%), *Enterococcus spps.* (12.9%), *coagulase-negative staphylococci* (CoNS, 5.9%), *Streptococcus spp*s (1.8%), and others

(8.8%). These bacteria presented a global-sensitivity rate of less than 30% to the commonly prescribed antibiotics.[29]

The study conducted in Uganda on drug sensitivity patterns of bacterial isolates from septic post-operative wounds. Pathogenic bacteria were recovered from 58.5% of the specimens. The isolates were*: S.aureus* (45.1%), *Coliforms* (16.9%), *Proteus mirabilis* (11.3%), *P.aeruginosa* (9.9%), *Klebsiella pneumoniae* (7.0%) and *Enterobacter spp* (2.82%). Most of the organisms were sensitive to gentamicin, ciprofloxacin and ceftazidime. There was resistance to ampicillin, amoxycillin and chloramphenicol. *Staphylococcus aureus* was generally sensitive to gentamicin (87.5%), ciprofloxacin (68.7%) and methicillin (75%), but resistant to erythromycin (56.2%) and ampicillin (97%). Most of the gram-negative bacteria isolated were sensitive to Ciprofloxacin, Gentamicin and Ceftazidime but resistance to Ampicillin, Amoxycillin and Chloramphenicol. Methicillin resistant *Staphylococcus aureus* (MRSA) strains formed 25% of this species. *Pseudomonas aeruginosa* was sensitive to gentamicin (87.5%) and ceftazidime (85.7%) but showed resistance to ciprofloxacin (57.2%). Some organisms e.g. *S.aureus, Pseudomonas aeruginosa* and *Proteus mirabilis* exhibited multi-drug resistance to the antibiotics tested. [30]

A Cross-sectional, prospective study conducted by Tigist et al indicated that out of 114 burn wound pus sample, 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 G. All isolates were found to be multi drug resistant, and one isolate was resistant to all the tested drugs.[31]

According to a study aimed at assessing bacteriology and antibiogram of pathogens from wound infections at Dessie, North East Ethiopia, Out of 599 wound swab samples analyzed, 422 (70.5%) were culture positive. Seventy eight (18.5%) of the culture had double infections. *Staphylococcus aureus* was the most frequently isolated pathogen which accounted for 208 (41.6%) of isolates followed by *Pseudomonas* spp. 92 (18.4%), *E. coli* 82 (16.4%), *Proteus* spp. 55 (11.0%), *Enterobacter* spp. 21 (4.2%), and *Citrobacter* spp. 21 (4.2%), *Klebsiella* spp. 12 (2.4%) and *Coagulate negative staphylococcus* (1.8%). Amoxicillin had the highest resistance

rate 78.9%, followed by tetracycline 76.1% and erythromycin (63.9%). The sensitivity rates of norfloxacin, ciprofloxacin and gentamicin were 95.1%, 91.8% and 85%, respectively. The overall multiple antimicrobial resistances rate was 65.2% and only 13% of the isolates were sensitive to all antimicrobial agents tested. The most frequently isolated bacteria were sensitive to ciprofloxacin, gentamicin, cloxacillin and norfloxacin. [26]

Cross-sectional prospective study was conducted to determine the bacteriology of open fracture wounds at Black Lion Hospital, Addis Ababa Ethiopia. A total of 162 bacterial pathogens were isolated from the 200 open fracture wounds sampled. *S. aureus* was the dominant isolate (14.8%) followed by *Acinetobacter* spp. (11.4%). Of the culture-positive wounds, 51.2% showed mono-microbial growth (single bacterial type) and 48.8% showed polymicrobial growth. The gram-positive and -negative bacteria accounted for 34.0 and 66.0%, respectively ($p < 0.05$). All gram-positive bacterial isolates showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and penicillin to which they showed intermediate level of resistance (60 - 80%). Most gram-positive isolates, 29/55 (52.7%) showed multiple drug resistance (resistance to three or more drugs). All gram negative bacterial isolates showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and amoxicillin (60 - 80%, intermediate level resistance). Fifty-one percent of the gram negative bacterial isolates were identified as multiple drug resistants (MDR).[32]

A Hospital based cross-sectional study was conducted on 322 wound samples at Jimma University Specialized Hospital, Ethiopia. The overall MDR among gram positive and gram negative bacterial isolates were (77%) and (59.3%) respectively. About, 86.2% *S.aureus* and 28.6% of Coagulase negative Staphylococci became MDR. Nearly 30.1% of *S.aureus* was resistant to six classes of antimicrobials. The average MDR rate of *Proteus, Klebsiella,* and *Providencia species* was 74.8%, 69.6% and 75% in that order. Nearly, 30.8% of *Proteus sp,* 32.6% of *Klebsiella sp* and 61% of *Citrobacter sp* were resistance to 4 classes each. Surprisingly, the average MDR rate for *Citrobacter sp* was 100%. About (76.7%) of *S.aureus* was oxacillin/methicillin resistant while (16.4%) were vancomycin resistant. *Proteus species* was the predominant isolates (27.9%) followed by *P. aeruginosa* and *S. aureus* (19.3%) and (19%) respectively.[12]

A retrospective study was conducted in Gondar on patterns and multiple drug resistance of bacteria pathogens isolates from wounds infection. Bacterial pathogens were isolated from 79 patients showing an isolation rate of 52%. *S. aureus* was the predominant species 65% followed by *E. coli* (10%), *Klebsiella pneumonia* 9%, *Proteus species* 4% and *Streptococci species* 4%. Amoge gram positive bacteria *S. aureus* shows high level of drug resistance against pencilline 59%, tetracycline 57%, ampicillin 55% and co-trimoxazole 35%. *E.coli* was found to be resistant to ampicillin in 87%, tetracycline also in 87% and co-trimoxazole 63%. The overall multidrug resistance pattern were found to be 78.5%.[33]

5. Objectives

5.1. General objectives

- To determine the prevalence, bacterial profile and drug resistance pattern of wound infection at Armed Force Referral and Teaching Hospital.

5.2. Specific objectives

- To determine the prevalence of bacterial wound infection at Armed Force Referral and Teaching Hospital
- To describe the bacterial pathogens responsible for the wound infection
- To determine the antimicrobial resistance pattern of commonly isolated wound microbes

6. Materials and Methods

6.1 Study area

The study was conducted at Armed Force Referral and Teaching Hospital which is located in Ledeta sub city, Addis Ababa, Ethiopia. It organized under Health Main Directorate, Ministry of Defense. It provides medical service to members of the Ethiopian defense force and their family ARTH has 15 wards with 600 beds, there are 378 health care professionals with different levels and filed of training. Based on the 2011/2012 annual report the hospital provides service for 100,005 outpatients and 4229 inpatient as well as 962 deliveries and 295,549 laboratory investigations. Other than patient diagnose, ARTH also engage in different activities like health teaching and research.

6.2. Study design and study period

A hospital based cross-sectional study was conducted from December 2012 to May 2013 at Armed Force Referral Teaching Hospital.

6.3. Population

6.3.1 Source Population

All patients who visited Armed Force Referral and Teaching Hospital during the study period.

6.3.2. Study population

All patients with wound who visited Armed Force Referral Teaching Hospital.

6.4. Variables of the Study

6.4.1. Independent variables:

- Age
- Sex

6.4.2. Dependant variables:

- Bacterial isolates
- Drug resistance pattern

6.5. Inclusion and exclusion criteria

Inclusion criteria

- Patients with wound infection
- patients agreed to participate and give informed consent

Exclusion criteria

- Patients who do not develop wound infection based on clinical examination during the study period was excluded from the study.

6.6 .Sample size determination and Sampling

6.6.1. Sample size determination

The sample size was calculated based on single sample size estimation. The value of p taken as 70.5% (0.705) from the previous study conducted on Bacteriology and antibiogram of pathogens from wound infections at Dessie, North East Ethiopia.[26] Considering 95% confidence interval, 5% margin of error and 70.5 proportion, the sample size was calculated using the following standard formula.

The sample size $n= z (_{\alpha/2})^2 p (1-p)/d^2$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value (α = 0.05) =>$Z_{\alpha/2} = 1.96$

p = Proportion of occurrence of the event to be studied 9% (0.09)

d = Margin of error at (5%) (0.05)

$n = (1.96)^2 0 .705(1-0.705)/(0.05)^2$

n = 319

A minimum sample size for this study was 319. But it was possible to attain only 300 samples within a given time frame.

6.6.2. Sampling procedures

Consecutive sampling technique was employed to include study participants who met the inclusion criteria.

6.7. Data collection procedures

Predesigned and structured questionnaire was developed and used for collection of data on socio-demographic characteristics(age and sex) of the patient.

6.7.1. Specimen collection and transportation

Wound beds were prepared before specimen collection by using Levine's technique [34] where the wound surface was cleansed of surface exudates and contaminants with a moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with non bacteriostatic sterile normal saline after removing the dressing. This technique is believed to be the best technique for swabbing open wounds and more reflective of tissue bioburden than swabs of exudate or swabs by other techniques .Cleansing the wound prior to obtaining swab specimens was done in an effort to remove immediate surface contaminating organisms (bacteria). The culture was more likely to represent the microbiology in the deep wound compartment. [34, 35] As part of Levine's technique, the end of a sterile cotton-tipped applicator was rotated over 1 cm^2 area for 5 second with sufficient pressure to express fluid and bacteria to surface from within the wound tissue.[34] double wound swabs were taken from each wound at a point in time to reduce the chance of occurrence of false-negative cultures During the study period a total of 300 different types of wound samples was collected. Following collection, wound specimens were transported to microbiology laboratory within 30 minutes by placing the swabs in to the sterile test tubes having 0.5 ml of sterile normal saline solution.(annex 1)

6.7.2. Specimen analysis

Culture and gram staining

All wound specimens were inoculated on blood agar (for gram-positive bacteria), and MacConkey agar (for gram-negative bacteria) (Oxoid, Ltd.,England). The plates were incubated in aerobic and microaerophilic atmosphere at 37°C for 24 - 48 h. Candle jar was used for microaerophilic atmosphere. Positive cultures were identified by their characteristic appearance

on their respective media, gram staining reaction and confirmed by the pattern of biochemical reactions using the standard method .[36]

Biochemical test

Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests. (Oxoid, LTD). Namely, carbohydrate utilization tests, indole production, urease test, ,manitol citrate utilization, lysine iron agar, oxidase test, Kligler iron agar (KIA),H_2S production,and motility.test. Gram-positive cocci were identified based on their gram reaction, coagulase and catalase test result.[36] (annex1)

Antibiotic susceptibility test:

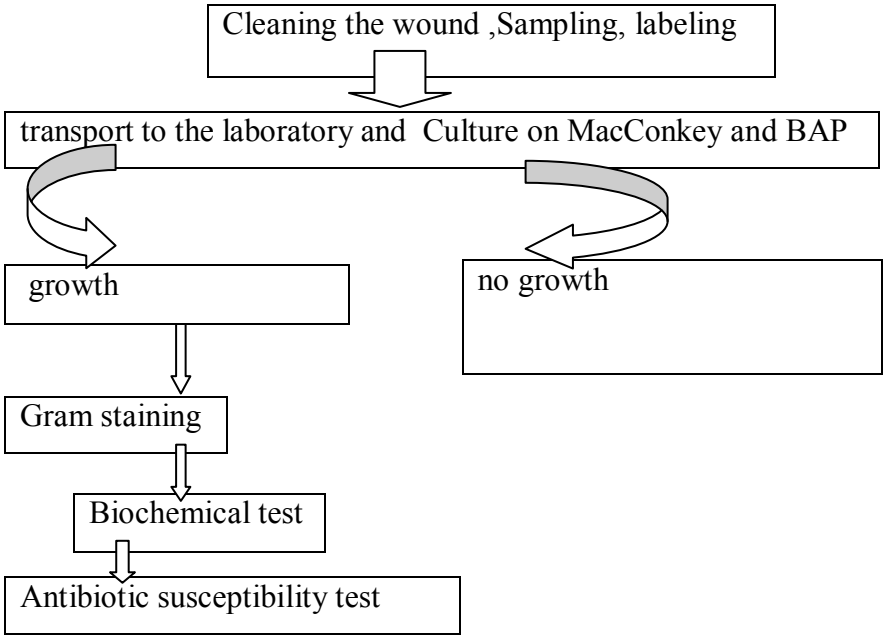
Antimicrobial susceptibility testing was performed for all isolates by using Modified Kirby Bauer disk diffusion method.[37]From a pure culture, 3 - 5 selected colonies of bacteria were taken and transferred to a tube containing 5 ml nutrient broth and mixed gently until a homogenous suspension was formed. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. A sterile cotton swab was used and the excess suspension was removed by gentle pressing and rotation of the swab against the inside wall surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar and blood agar (Oxoid, , Hampshire, England). Mueller-Hinton agar was used for all gram negative and -positive bacteria, except for *Streptococci spp*. The sensitivity test of *Streptococci* was performed on Mueller-Hinton agar with 5% sheep's blood.

The drugs tested were in the following concentrations: amoxicillin (AML) (25 µg), ampicillin (AMP) (10 µ g), penicillin G (P), oxacilin (ox) (1µg), ceftriaxone (CRO), cefoxitine (FOX)(30 µ g), cefotaxim(CTX)(30 µ g) chloramphenicol (C) (30 µ g), clindamycin (DA) (2 µ g), cloxacillin (OB) (5 µg), erythromycin (E) (15 µ g), gentamicin (CN) (10 µ g), , norfloxacin (NOR) (10 µ g), (10 units), tetracycline (TE) (30 µ g), and trimethoprim-sulphamethoxazole (SXT) (25 µ g).

Only the conventional antibiotics regularly available for frequent use in the study area were considered for this study and all the disks used for the test were from (Oxoid Ltd. England). All

Gram positive bacteria were tested against amoxicillin, penicillin G, clindamycin, ampicillin, ceftriaxone, cefotaxim, chloramphenicol, cloxacillin, erythromycin, gentamicin, oxacilin norfloxacin, tetracycline, trimethoprimsulphamethoxazole. All gram-negative bacteria were tested against amoxicillin, ampicillin, ceftriaxone, Cefotaxim, chloramphenicol, gentamicin, norfloxacin, tetracycline, and trimethoprim-sulphamethoxazole. Diameters of the zone of inhibition around the disc were measured using a ruler in millimeters, and bacterial strains ware classified into three groups: as sensitive, intermediate, and resistant according to the standardized table supplied by the CLSI [38]

Work flow



6.8. Data management and Quality control

Data quality was ensured through use of standardized data collection materials, proper training before the start of data collection and intensive supervision during data collection by the principal investigator. For laboratory analysis Pre-analytical, analytical and post-analytical stages of quality assurance that is incorporated in Standard operating procedures (SOPs) of the microbiology laboratory of ARTH was strictly followed. In addition, well-trained and experienced laboratory professionals were participate in the laboratory analysis procedure.

Pre-analytical phase

Pus swabs were aseptically obtained using sterile cotton from wound sites before the wound is cleaned by antiseptic solution. Following collection from patients, specimens was transported by placing swab in a sterile test tube to the microbiology laboratory within 30 minutes. 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 sample container was used.

Analytical phase

All materials, equipment and Procedures were adequately controlled. All stains and reagents were clearly labeled, dated, and stored correctly. The preparation, fixation, staining and reporting of smears as detailed in the SOPs of the microbiology laboratory of ARFTH was strictly followed At regular intervals and whenever a new batch of gram stain is prepared, control smears of appropriate organisms were stained to ensure correct staining reactions.

For each item of equipment there was clear operating and cleaning instructions, and service sheets. The operating temperature of a refrigerator, incubator, and water-bath was monitored and documented .Culture media was tested for Performance and sterility. To standardize the inoculums density of bacterial suspension for the susceptibility test, a 0.5 McFarland standard was used and standard reference strain *S. aureus* (ATCC-25923), *E. coli* (ATCC-25922) and *P. aeruginosa* (ATCC-27853) was used as Control bacteria strains. All the strains were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

Post-analytical phase

The results were recorded with the patients' identification number. The terminology and format used in reporting was standardized. All reports were concise and clearly presented. Before leaving the microbiology laboratory, all reports were double checked for correctness.

6.9. Data Processing and Analysis

Data entry and analysis was done using SPSS statistical software version 20. The descriptive statistics was calculated & logistic regression analysis was used to see the relation between dependent variable and independent variables. The level of significance was set at 0.05 in order to consider a p-value < 0.05 as indicator of a statistically significant difference with 95% confidence interval.

6.10. Ethical consideration

This research project was approved by "Departmental Ethics and Research committee" of the Department of Medical Laboratory Sciences, Collage Health Science, School of Allied Health Science of Addis Ababa University. Permission was obtained from the ARTH administrator. Subjects were recruited after they become informed about the objectives and use of the study and after they give informed consent. There was minimal risk associated with the process of sampling; it is the same as taking specimen for culture and sensitivity in the routine laboratory. For all confirmed wound infections the responsible clinician of the subjects was informed. All the information contained within the study was kept confidential.

7. Result

Demography

A total 300 patients with wound infection were included in this study, out of which, 268(89.3%) were male and 32(10.7%) were female. resulting in an overall male to female ratio of 1:8.4.The age of the patients ranged from 10year to 68 years, with mean age of 30 [SD=11.5] years. The mean age of male and female patients was [(30.(SD=11.2) and 33.8 (SD=13.2)] years, respectively. The infection rate was higher in male (87.3%) than female (12,7%) but the difference was not statistically significant (P=0.103). The infection rate was relatively high (49.3%) in the age group of 21-30 years old followed by 31-40 years of age group (20.0%).
The age and sex distribution of patients involved in this study is presented in Table (Table1)

Table 7.1: Age and sex distribution of patents with wound infections at AFRTH from December 2013 to May 2014

Demographic characteristics	No. (%) of culture positive	No. (%) of culture negative	P-value
Sex Male	179(87.3)	89 (93.7)	0.103
Female	26(12.7)	6 (6.3)	
Total	205(100)	95(100)	
Age groups (years)			
10-20	17 (8.3)	18(18.9)	
21-30	101(49.3)	54(56.8)	0.408
31-40	41(20)	12(12.6)	0.123
41-50	30 (14.6)	9(9.5)	0.309
>51	16(7.8)	2(2.1)	0.094
Total	205(100)	95(100)	

Etiology of wounds

Out of 300 samples, 205(68.3%) samples were culture positive while 95 (31.7%) of wound swab cultures showed no growth. Out of 205 positive samples 33 (16.1%) had double infection and total 238 bacteria were isolated from 205 cases. 102(42.9%) were Gram positive while the rest 136 (57.1%) were Gram negative. *Staphylococcus aureus* 91 (38.2%) was the most frequently isolated Gram positive bacteria where as *pseudomonas aeroginesa* 53 (22.3%) was the most frequently isolated Gram negative bacteria. *Streptococcus spps* was the least prevalent etiologic agent. The proportion of each bacterial isolate to the total number of isolates is presented in Table 2.

Table 7.2: Type and frequency of pathogens isolated from wound infections at AFRTH from December 2013 to May2014.

Bacteria Isolated	Number	percent
Staphylococcus aureus	91	38.2
Pseudomonas aeroginesa	53	22.3
Proteus spp.	24	10.1
Klebsiella spp	21	8.8
E.coli	19	8.0
Entrobacter spp.	9	3.8
CoNS	10	4.2
Citrobacter	8	3.4
Providencia spp	2	0.8
Streptococcus spp.	1	0.4
Total	238	100

Key CoNS= Coagulase negative staphylococci

All double infections in our study involved Gram positive and Gram negative bacteria with *S. aureus* and *Pseudomonas aeroginesa show* the most common association in 15 (45.5%) cases. Infections with *S. aureus* and *E. coli*, *S. aureus* and *Proteus* spp, *Klebsiella spp*. and *CoNS*,*S.*

aureus and *Enterobacter spp*. with rates of 6(18.2%), 6(18.2%) ,5(15.2%) and 1(3%) respectively were among the double infections isolated in this stud.

Table 7.3.Type and frequency of pathogens isolated from wound infections at **AFRTH from December 2013 to May2014.**

Pathogens	Frequency	Percentage
S. aureus and *Pseudomonas spp.*	15	45.5
S. aureus and *E.coli*	6	18.2
S. aureus and *Proteus* spp.	6	18.2
Klebsiella spp. and *CoNS*	5	15.2
S. aureus and *Enterobacter spp*.	1	3
Total	33	100

Key: *CoNS* = Coagulase negative staphylococci

Antimicrobial resistance pattern

The resistance patterns of bacteria isolated from wound infection are presented in Table 7.4&7.5. Among Gram positive bacteria, high level of resistance was observed against Penicillin G (86.3%), and ampicillin (67.6%). Likewise The least effective antibiotic for Gram Negative were Ampiciline(87.5%) and amocilline (83.8%)

Analysis of species specific resistance rates indicated that most of *S. aureus* was mostly resistant to Penicillin G (91.2%),Ampcillin (73.6) and tetracycline (67%). On the other hand, *S. aureus* was susceptible to, Ceftriaxone, norflaxocin, and Cefotaxim with resistance of only 7.7%,7.7%, and 9.9% , respectively. Similarly, 50% of CoNS was resistance to penicillin G. Fortunately, *Streptococcus spp.* was 100% sensitive to many of the antimicrobial drugs tested. (Table 7.4)

Table 7.4 Antimicrobial drugs resistance pattern of gram positive bacteria identified from wound infection at AFRTH from December 2013 to May 2014

Drugs No (%) resistance to															
Bacteria	CTX	CRO	CN	NOR	TE	C	OX	APL	AML	OB	P	E	SXT	CD	Average (%)
S.aureus N=91	9 (9.9)	7 (7.7)	13 (14.3)	7 (7.7)	61 (67)	24 (26.4)	55 (60.4)	67 (73.6)	50 (54.9)	42 (46.2)	83 (91.2)	29 (31.9)	18 (19.8)	38 (41.8)	39.5
CoNS N=10	1 (10)	1 (10)	-	-	2 (20)	3 (30)	2 (20)	2 (20)	5 (50)	1 (10)	5 (50)	-	-	-	15.7
Streptococcus spp. N=1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*0*

Key: OX = Oxacillin, , OB = Cloxacillin, E = Erythromycin, CD = Clindamycin, P = Penicillin G APL = Ampicillin, CRO = Ceftriaxone, NOR = Norfloxacin, CTX = Cefotaxim, CN = Gentamycin, C = Chloramphenicol, TE: Tetracycline; SXT: Trimethoprim-sulphamethoxazole, AML: Amoxicillin, *CoNS* = Coagulase negative staphylococci

Pseudomonas aeroginesa showed the highest resistance to Amoxillin (83%), Ampcillin(84.9%) and Chloramphenicol (62.5%) while norfoxacilin and Gentamaycine were the most effective antibiotics with resistance rates of 5.7% and 11.3%. respectively. Out of 24 isolates of *Proteus*

spp, (95.8%), (87.5%), and (62.5%) were resistant to Ampicillin, Amoxicillin, and tetracycline respectively. *Klebsiella SPP.* demonstrated high level of resistance to Amoxillin (90.5%) and ampicillin 85.7% Furthermore, *E.coli* showed 78.9% resistance to Ampcillin and 84.2% resistance to amoxillin. whereas *E.coli* were sensitive to Gentamaycine, ceftraxone and norfoxacilin with 5.3% resistance rates for each them.

Table 7.5 Antimicrobial drugs resistance pattern of gram negative bacteria identified from wound infection at AFRTH from December 2013 to May 2014

Drugs No (%) resistance to										
Bacteria	CTX	CRO	CN	NOR	TE	C	AMP	AML	SXT	Average (%)
*Pseudomonas aeroginesa .(*N=53)	12 (22.6)	7 (13.2)	6 (11.3)	3 (5.7)	30 (56.6)	33 (62.5)	45 (84.9)	44 (83)	27 (50.9)	43.4
Proteus spp. (N=24)	1 (4.3)	1 (4.2)	6 (25)	2 (8.5)	15 (62.5)	12 (50)	23 (95.8)	21 (87.5)	8 (33.3)	41.2
Klebsiella spp.(N=21)	6 (28.6)	6 28.6)	6 (28.6)	2 (9.5)	12 (57.1)	7 (33.3)	18 (85.7)	19 (90.5)	5 (23.8)	42.8
E coli(N=19)	2 (10.5)	1 (5.3)	1 (5.3)	1 (5.3)	10 (52.6)	4 (21.1)	15 (78.9)	16 (84.2)	5 (26.3)	32.2
Enterobacter spp.(N=9)	2 (22.2)	2 (22.2)	1 (11.1)	-	4 (44.4)	3 (33.3)	8 (88.9)	6 (66.7)	2 (22.2)	34.6
*Citrobacter(*N=8)	2 (25)	2 (25)	2 (25)	-	4 (50)	6 (75)	8 (100)	6 (75)	1 (12.5)	34.7
Providencia spp.(N=2)	-	-	-	-	1 (50)	-	2 (100)	2 (100)	-	16.7

Key: APL = Ampicillin, CRO = Ceftriaxone, NOR = Norfloxacin, CTX = Cefotaxim, CN = Gentamycin, C = Chloramphenicol, TE: Tetracycline; SXT: Trimethoprim-sulphamethoxazole, AML: Amoxicillin,

Multi drug resistance of the isolates

Table7.6; show multi drug resistant of the gram positive bacteria. The overall rate of MDR (resistant to three or more antibiotics) among gram positive isolates was 73.6%. out of the total of 91 *S.aureus* isolate tested 72(79.2%) were resistant to three or more antibiotics while only 3.3% of the isolate showed no resistance to all the antibiotics tested. 3(30%) of *CoNS* were resistance to three or more antibiotics.

Table 7.6: Multidrug resistance of gram positive bacteria identified from wound infection at AFRTH from December 2013 to May 2014

Number of antimicrobial resisted to No (%									
Bacteria	**R_0**	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_{6-12}**	**Total**	**MDR**
S.aureus	3(3.3)	5(5.5)	11(12.1)	5(5.5)	8(8.8)	16(17.6)	43(47.3)	91	72(79.2)
CoNS	-	1(10)	6(60)	2(20)	1(10)	-	-	10	3(30)
S.pyogenes	1(100)	-	-	-	-	-	-	1	0
Total	4(3.9)	6(5.9)	17(16.7)	7(6.9)	9(8.8)	16(15.7)	43(42.2)	102	75(73.6)

Key *CoNS* = Coagulase negative staphylococci; R_0 = no resistance to antibiotic ,R_1= resistance to 1 antibiotics. R_2= resistance to 2 antibiotics R_3=resistance to 3 antibiotics, R_4 =resistance to 4 antibiotics, R_5 = resistance to 5 antibiotic, R_{6-12}=resistance to 6-12antibiotics

The overall MDR (resistant to three or more antibiotics) rate of gram negative bacteria was 67.6%. Relatively higher rate of MDR was seen among *Citrobacter*, ,*Proteus Pseudomonas* and *Klebsiella* accounting 87.5%, 75% ,67.9% and 66.6% respectively.

Table 7.7: Multidrug resistance of gram negative bacteria identified from wound infection at AFRTH from December 2013 to May 2014

Number of antimicrobial resisted to No (%									
Bacteria	**R_0**	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_{6-9}**	**Total**	**MDR**
Pseudomonas aeroginesa	2(3.8)	1(1.9)	14(26.4)	6(11.3)	8(15.1)	10(18.9)	12(22.6)	53	36(67.9)
Proteus spp.	-	-	6(25)	5(20.8)	4(16.7)	6(25)	3(12.5)	24	18((75)
Klebsiella spp.	-	1 (4.8)	6(28.6)	1(4.8)	5(23.8)	4(19)	4(19)	21	14(66.6)
E.coli	-	1(5.3)	7(36.8)	7(36.8)	1(5.3)	3(15.8)	-	19	11(57.9)
Entrobacter Spp.	-	1(11.1)	3(33.3)	_	4(44.4)	1(11.1)	_	9	5(55.5)
Citrobacter	-	-	1(12.5)	2(25)	4(50)	-	1(12.5)	8	7(87.5)
Providencia spp.	-	-	1(50)	1(50)	-	-	-	2	1(50)
Total	2(1.5)	4(2.9)	38(27.9)	22(16.2)	26(19.1)	24(17.6)	20(14.7)	136	92(67.6)

Key R_0 = no resistance to antibiotic ,R_1= resistance to 1 antibiotics. R_2= resistance to 2 antibiotics R_3=resistance to 3 antibiotics, R_4 =resistance to 4 antibiotics, R_5 =resistance to 5antibiotic , R_{6-9}=resistance to 6-9 antibiotics

8. Discussion

A prevalence of 68.3% wound infections was observed in this study. This was higher than that previously reported in Gondar with a prevalence of 52% [33],Nigeria 64.8 %[9] and Indian 47%.[39] However, the prevalence observed in this study is lower than that previously observed in Jimma (96.3%)[12],Dessie (70.5%) [26] ,Nigeria (90%)[40] and Italy (90.8%)[28] Different factors related to wound bed preparation; sample collection, sample transportation and culturing technique might have an effect in the reduction of the bacterial isolation rate.

Staphylococcus aureus and *Pseudomonas aeroginesa* were the first and second most prevalent bacterial agents isolated in this study. This observation was in agreement with several previous studies conducted at different places.[26,9,31] However, Girma.et al reported Proteus species as the most prevalent agent for wound infections. Variation in the distribution of microbial agents even between different geographical locations and regions within the same country may be responsible for this diversity .[9] The possible reason for the high frequency of *Staphylococcus aureus* and *Pseudomonas aeroginesa* is that these bacteria commonly found in the hospital environment[12] which might increase wound infection rate and cross contamination among admitted patients. In addition these bacteria are normal flora in healthy person(especially *S. aureus* on skin) when they get breaks on skins and soft tissue they can easily disseminate as it was explained by Khana et al.[41]

The prevalence of mixed infections (16.1%) observed in this study was lower than the 18.5%,[26] 22.9%[12] and 33.2%[9] reported in previous studies. Difference in identification methods are known to influence the relative prevalence of bacteria which makes comparison of results difficult.

S.aureus showed an average resistance rate of 39.5% to most of the antimicrobial drugs tested which is lower than the previous studies done locally by Mulu et al [42] and Girma et al [12] where average resistance of 52%, and 54.1% were obtained for the commonly used antibiotics. About 60% of S.aureus was oxacillin/methicillin resistant (MRSA). which is lower than (76.7%) resistant reported by Grima et al [12] and 77.3% resistant by tigist et al. [31] And yet 60% was higher than the finding of of Amare et al., in Ethiopia [27], JR Anguzu in Uganda[30] and A.

Giacometti[28] in Italy where 34.6%, 25% and 54.4%%MRSA were reported respectively. In addition *S. aureus* showed least resistance to gentamicin (14.3%), cefotaxim (9.9%) ceftriaxone, (7.7%)and norfloxacin (7.7%). comparable finding where obtained from other study.[32,26] similarly majority of *CoNS* isolate were sensitive for, norfloxacin, gentamicin, erythromycin, clindamycin, which is in line with the previous studies reported from the some country .[12,32]

Pseudomonas aeroginesa showed an average resistance rate of 43.4% This finding agrees with previous studies done elsewhere in Ethiopia, which report average resistance rate (39.9%)[12.] In this study pseudomonas showed high resistant to ampicillin (84.9)% and amoxicillin (83%) where as other studies report 100% resistant for both drugs.[27] Lower rate of resistance to ceftriaxone, (13.2%) gentamicin (11.3%) and Norfloxacin (5.7)% were documented in this study. This result is consistent with the data obtained by yishak et al [32] and Girma et al [12] . But 100% resistance for ceftriaxone where reported by other study [27]which is much higher than the current study.

In this study, Proteus *spp.* conferred high resistant to ampicilin(95.8%) amoxicillin(87.5%) and teracyclin (65.5%),with average resistance rate of 41.2% for all tested drug which agrees with similar level of resistance reported in other studies. [12,26] *Proteus sps* show low label of resistance to cefotaxim (4.3%) ceftriaxone (4.2%) and norfloxacin (8.5%) .Comparable finding were reported by Girma et al [12]

The present study documented that *Klebisela spp*. show lower rate of resistant to norfloxacin (9.5%) which is higher than 0% resistant reported by Yishak et al.[32] where as both studies report similar resistance rate of *Klebisela spps* for trimethoprim-sulphamethoxazole. The average resistance rate for *Klebisela spps* in this study was 42.8% which agree with average resistance of 47.3% reported by other study [12]

The average resistance rate of *E. coli i*n this study was 32.2%. comparable result was reported by other study.[12] the resistance of *E coli* for cefotaxim and ceftriaxone was 10.5% and 5.3% respectively which was much lower than 66.7%[12,26] of resistance for both drugs and 55.6% of

resistance for ceftriaxone[27] reported by previous studies. ceftriaxone ,gentamycin and Norfloxacin found to be more effective drugs for *Ecoli* with only 5.3% resistance for each of them. Comparable finding were reported by Yisak et al.[32] with 11.8% resistance for ceftriaxone and gentamycin and 5.9% resistance for norfloxacin .

*Enterobacter spp*s. showed low resistance to most of antimicrobial tested with 0% resistance to Norfloxacin which is the exact agreement with the resistance level recorded in a study performed by Yisak.[32] the average resistance of *Enterobacter spp* in this study was 34.6% and high resistance was recorded for ampiciline (88.9%) and amoxicillin (66.7%) but which is lower than 100% resistance to amoxicillin reported by other studies.[26, 27]

In this study *Citrobacter* maximum resistance was conferred to ampicilin(100%), chloraphinichol(75%) and amoxicillin(75%) which is comparable to the result reported Girma et al.[12] in contrast other study report 66.7% resistance for ampicilin and 16.7% resistance for both chloraphinichol and amoxicillin.[32]

Most of Gram negative bacterial isolates showed low level of resistance to all antibiotics tested Except for ampicillin and amoxicillin. This might be due to these antibiotics are the most commonly used antibiotics and resistant pattern were reported from many studies.[12, 27, 26, 30]

In this study, the overall MDR (resistance to three or more drugs) rate of gram positive isolates was 73.8% which agree with 77% MDR reported by Girma et al[12.] But which is higher than 52.7% MDR rate reported by Yisak et al.[32] and lower than 100% MDR reported by Mulu et al .[42] 79.2% *S.aureus* and 30% of *CoNS* were resistant to three or more antibiotics. In similar study 86.2% and 28.6% MDR rate were documented for these two groups of bacteria respectively. [12]

MDR (resistance to three or more drugs) rate of gram negative bacteria in this study was 68.5%. This finding was slightly higher than 59.3% and 51.4% of MDR rate reported by Girma et al[12] and Yisak et al.[32] but which is much lower than 95.5% resistance reported by Mulu et al.[42]higher rate of MDR was seen among *Citrobacter,,Proteus, Pseudomonas* and *Klebsiella* accounting

87.5%, 75% ,67.9% and 66.6% respectively which agree with previous study conducted in Jimma.[12] where 100% MDR for Cirtobacter, 74.8 %MDR for *Proteus,* and 69.6% MDR for *Klebsiella spp* .were reported.

9. Limitation of the study

- It was not possible to include anaerobic bacteria due to poor laboratory facilities constraints.

10. Conclusion and recommendation

Conclusion

The prevalence of wound infection in this study was 68.3%. Out of 205 positive samples 33 (16.1%) had double infection and total 238 bacteria were isolated from 205 cases. 42.9% were Gram positive while the rest 57.1% were Gram negative. *Staphylococcus aureus* was the most frequently isolated Gram positive bacteria where as *Pseudomonas aeroginesa* was the most frequently isolated Gram negative bacteria. All double infections in our study involved Gram positive and Gram negative bacteria with *S. aureus* and *Pseudomonas* show the most common association in 45.5% cases.

Gentamicin ,norfloxacin and ceftraxone were the most effective drugs against the tested gram-positive and - negative bacteria, where as penicillin G and ampicillin were the least effective antibiotics against gram positive bacteria isolates similarly amoxicillin and ampicillin were the least effective antibiotics against gram negative bacteria isolates .

The overall MDR (resistant to three or more antibiotics) rate of gram positive and gram negative bacteria isolates were 73.6% and 67.6% respectively.79.2% of *S.aureus* were resistant to three or more antibiotics . Among gram negative bacteria relatively higher rate of MDR was seen in Citrobacter, *Proteus, Pseudomonas* and *Klebsiella species,*

.

Knowledge of the microbial flora of wound and the resistance pattern are important tools in the management of wound and are also useful in formulating rational antibiotic policy

Recommendation

- In future ,the prevalence and drug susceptibility pattern of wound infections should be done by including anaerobic bacteria, fungus and other micro-organism those can be important causes of infections
- Empirical treatment to wound infections may provoke drug resistance; therefore treatment should be based on the result of culture and sensitivity.
- In the absence of laboratory test, It is recommended that norfloxacin gentamicin and ceftraxone can be used in preference to other commonly used antibiotics in the area.
- There is need for hospital to encourage periodic review of the microbial flora wound and the antibiotic sensitivity pattern.

11. Reference

1.Nitin Goel Insan, Nikhil Payal, Mahesh Singh, Amod Yadav, B.L Chaudhary. Post operative wound infection: Bacteriology and antibiotic sensitivity pattern. *Int J Cur Res Rev*.2013;05 (13):74-79.

2.Esebelahie, N. O. Newton-Esebelahie, F. O. Omoregie R. Aerobic bacteria isolated from infected wound. *African journal of clinical and expremental microbiology*. 2013;14(3):1595-689.

3.Mohammad Shahid Raza, Anil Chander, Abirodh Ranabhat. Antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in a Tertiary Care Hospital, Kathmandu, Nepal. *Open Journal of Medical Microbiology*.2013;3:159-163.

4.Mordi R. M. and Momoh M.I.Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *Afr.J. Biotechnol.*.2009;8(5): 725-730.

5.Bowler PG, Duerden BI, Amstrong DC. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev.* 2001;14:244-269.

6.Rubin, R.H. Surgical wound infection: epidemiology, pathogenesis, diagnosis and management. *BMC Infect. Dis*.2009;6: 171-172.

7.Sani R. A., Garba S. A., Oyewole O. A., Ibrahim A. Antibiotic Resistance Profile of Gram Positive Bacteria Isolated from Wound Infections in Minna, Bida, Kontagora and Suleja Area of Niger State .*Journal of Health Sciences* 2012; 2(3): 19-22.

8.Adegoke, Anthony A, Tom Mvuyo, Okoh Anthony I. and Jacob Steve. Studies on multiple antibiotic resistant bacteria isolated from surgical site infection. *Scientific Research and Essays*.2010;5(24):3876-3881.

9.Egbe CA, Omoregie R, Igbarumah IO, Onemu S. Microbiology of wound infections and its associated risk factors among patients of a Tertiary hospital in Benin City, Nigeria. *JRHS*. 2011;11(2):109-113.

10.Adenike A.O. Ogunshe, Mary T. Niemogha, Gabriel N. Azum & Anthony N. Odikagbue. Microbiological evaluation of antibiotic resistance in bacterial flora from skin wounds. *J Pharm Biomed Sci*.2012;22(6):1-7.

11. Livermore DM: Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? *Clin Infect Dis*.2002;34:634–64.
12. Girma Godebo, Gebre Kibru and Himanot Tassew. Multidrug-resistant bacteria isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. *Annals of Clinical Microbiology and Antimicrobials*.2013;12(17):1-7.
13. Harding K, Renyi R. The International Wound Infection Institute – a new global platform for the clinical management of infected wounds. *Int Wound J.* 2009; 6(3): 175-78.
14. Praveen Kumar Doddmani and Neelima. Bacteriological profile of surgical site infection. *Int J Pharm Bio Sci* .2013 ;4(3):217 – 221.
15. Anusha S, Vijaya LD, Pallavi K, Manna PK, Mohanta GP, et al. An. Epidemiological Study of Surgical Wound Infections in a Surgical unit of Tertiary care Teaching Hospital. *Indian Journal of Pharmacy Practice* .2010;3(4):8-12.
16. Chandan K. Sen, Gayle M. Gordillo, Sashwati Roy, Robert Kirsner, Lynn Lambert, et al. Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy. *Wound Repair Regen*. 2009 ;17(6):763–771.
17. Eli N. Perencevich, Kenneth E. Sands, Sara E. Cosgrove, Edward Guadagnoli, Ellen Meara, et al. Health and Economic Impact of Surgical Site Infections Diagnosed after Hospital Discharge. *Emerging Infectious Diseases*.2003;9(2):196-2003.
18. Suchitra Joyce B. and Lakshmidevi N. Surgical site infections: Assessing risk factors, outcomes and antimicrobial sensitivity patterns. *Afr. J. Microbiol. Res*. 2009;3(4):175-179.
19. Sepideh Bagheri Nejad, Benedetta Allegranzi, Shamsuzzoha B Syed, Benjamin Ellisc & Didier Pittetd. Health-care-associated infection in Africa: a systematic review. *Bull World Health Organ*.2011;89:757–765.
20. Taye M. Wound infection in Tikur Anbessa Hospital, surgical department. *EM J*. 2005; 43:167-74.
21. Gottrup F. A specialized wound healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. *Am J Surg.*2004;187(5):38S–43S.

22. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. *Circulation*.2005;111:2398–409.

23. I Rich, A. & McLachlan, L. How living with a leg ulcer affects people's daily life: a nurse-led study. *Journal of Wound Care.*2003; 12(2):51-54.

24. Theoklis E. Zaoutis. Antibiotic Resistance: Who Will Pay the Bills? *Clinical Infectious Diseases.* 2009; 49:1185–6.

25. Robert H. Demling, and Barbara Waterhouse. The Increasing Problem of Wound Bacterial Burden and Infection in Acute and Chronic Soft-Tissue Wounds Caused by Methicillin-Resistant*Staphylococcus aureus. J Burns Wounds*. 2007;16:1-19.

26. Mulugeta K. Azene and Bayeh A. Beyene .Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia. *Tanzania Journal of Health Research.*2011;13(4):1-10.

27. Amare B, Abdurrahman Z, Moges B, Ali J, Muluken L, et al. Postoperative Surgical Site Bacterial Infections and Drug Susceptibility Patterns at Gondar University Teaching Hospital Northwest Ethiopia. *J Bacteriol Parasitol*. 2011;2(8):126.

28. A. Giacometti, O. Cirioni, A. M. schimizzi, M. S. Del Prete, F. Barchiesi et al. Epidemiology and Microbiology of Surgical Wound Infections. *Journal of clinical microbiology*.2000;38(2): 918–922.

29. Ntsama Essomba C., Avomo J., Essomba R., Esiene A., Leme Banock L., et al. Isolation of bacteria in surgical site infections and determination of their sensitivity to commonly prescribed antibiotics .*Sky Journal of Microbiology Research*.2013; 1(6):40 – 46.

30. JR Anguzu and D Olila .Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *Afr Health Sci.* 2007;7(3): 148–154.

31. Tigist Alebachew, Gizachew Yismaw, Ayelegn Derabe, Zufan Sisay. Staphylococcus aures burn Wound infection among patients attending yekatit 12nts hospital burn unit, Addis Ababa, Ethiopia. *Ethiop J Health Sci*. 2012; 22(3): 209-213.

32. Yishak Abraham and Biruk L. Wamisho. Microbial susceptibility of bacteria isolated from open fracture wounds presenting to the err of black-lion hospital, Addis Ababa University, Ethiopia. *Afr. J. Microbiol. Res.*. 2009; 3(12):939-951.

33. Mulu A, Moges F, Tessema B, Kassu A: Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital. *Ethiop Med J*.2006, 44:125–131.
34. Levine NS, Lindberg RB, Mason AD Jr, Pruitt BA Jr: The quantitative swab culture and smear: a quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma*. 1976; 16:89–94.
35. Gardner SE, Frantz R, Hillis SL, Park H, Scherubel M: Diagnostic validity of semiquantitative swab cultures. Wounds. 2007,;19:31–38.
36. Cheesbrough M: District laboratory practice in tropical countries volume II: microbiology. Cambridge (UK): Cambridge University Press; 2006: 1–479.
37. Bauer A, Kirby W, Sherirs J, Turck M .Antibiotic susceptibility testing by standard Single disk method. *Am J Clin Pathol* .1996;45: 493-496.
38. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. *Twent Informational Suppl*. 2010; 29:1–160.
39. Sibor JO, Oseni A, Eyaufe A, Osagie R. Turay A. Incidence of aerobic bacteria and Candida albicas in post operative wound infections. *Afr J Microbiol Res*.2008;2:288-291.
40. Ezebialu CU, Chukwura EI. Ezebialu IU. Bacterial pathogen associated with wound infection at national orthopaedic hospital, Enugu. *Nigerian J Microbiol.* 2010;24(1):1987-1992
41. Khanal LK, Jha BK: Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) among skin infection cases at a hospital in Chitwan, Nepal. *Nepal Med Coll J* 2010; 12:224–228.
42. Mulu W, Kibru G, Beyne G, Damtie M: Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. *Ethiop JHealth Sci*. 2012: 22:1–1

Annex 1: Procedure for specimen collection and processing

I.Laboratory procedure for collection ,transportation and culturing of wound swab

1. Cleansing the wound with normal saline prior to obtaining swab specimens
2. Rotate sterile cotton-tipped applicator 1cm square area for 5 seconds with sufficient pressure to express fluid and bacteria to surface
3. placing the swabs in to sterile test tubes having 0.5 ml of sterile normal saline solution
4. Label the sample as soon as possible with the patient code number
5. transport the specimen to the laboratory at room temperature within 30 minutes of collection
6. Inoculate in to BAP and MacConkey agar aseptically
7. Incubate the inoculated blood agar plate at 35–37_C in a carbon dioxide atmosphere (candle jar) and the MacConkey agar plate aerobically.
8. Examine and report the culture; if the culture have growth ,look for colony characteristics perform gram reaction and biochemical test and determine drug susceptibility pattern to the isolated organism

II. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with patient code number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.
4. Fix the dried smear by using heat or chemicals (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranine stain for 2 minutes.
11. Wash off the stain with clean water.
12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.

13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram-negative bacteria ----------pale to dark red

III. Laboratory procedure for Biochemical testing

Biochemical tests for gram positive bacteria: Gram-positive cocci was identified based on their gram reaction, catalase and coagulase tests results.

Catalase test

Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non catalase producing.

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.

Procedure

1. pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution
3. look for immediate bubbling
4. interpretation :

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test

Controls

Positive coagulase control: *Staphylococcus aureus*

Negative coagulase control: *Escherichia coli*

Coagulase test

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

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

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

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

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

4. Clumping within 10 secs . . . . . *S. aureus*

No clumping within 10 secs . . . . . No bound coagulase

Controls

Positive coagulase control: *Staphylococcus aureus*

Negative coagulase control: *Escherichia coli*

If slide test is negative proceed to Tube test method

Tube test method (detects free coagulase)

Procedure

1.Take three small test tubes and label:

T _ Test organism (18–24 h broth culture)*

Pos _ Positive control (18–24 h *S. aureus* broth culture)*

Neg _ Negative control (sterile broth)*

2 Pipette 0.2 ml of plasma into each tube.

3 Add 0.8 ml of the test broth culture to tube T.

Add 0.8 ml of the *S. aureus* culture to the tube labeled 'Pos'.

Add 0.8 ml of sterile broth to the tube labeled Neg'.

4. After mixing gently, incubate the three tubes at 35–37 _C. Examine for clotting after 1 hour

If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again

Results

Clotting of tube contents or . . . . . *S. aureus*

fibrin clot in tube

No clotting or fibrin clot . . . . . Negative test

Biochemical test for gram negative bacteria:- Identification of gram negative bacteria was based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, KIA, lysine decarboxylase agar, manitol, urea agar and motility medium.
3. Incubate at 35-37 Oc for 18-24 hours
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the biochemical tests

IV. Laboratory procedure for Antimicrobial sensitivity testing

Procedure

1. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of nutrient broth
2. Match the turbidity of the suspension against the turbidity standard
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
5. Using a sterile forceps or needle ,place the antimicrobial disc on the inoculated plate
6. Within 30 minutes of applying the discs, invert incubate the plate aerobically at 35-37oC For 18-24 hours
7. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light
8. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.
9. Using the Interpretative Chart, interpret the zones sizes of each antimicrobial, reporting the organism as ‘Resistant’, ‘Intermediate’ and , ‘Susceptible

Annex 2: English version of participant information sheet and consent

I. Participant information sheet

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

Title of the Research Project: Bacterial profile and drug resistance pattern of pathogen isolated from wound infection at ARTH, Addis Ababa, Ethiopia

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

Background information

Background: Wound infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stay, profound discomfort and significant increased in healthcare cost. Therefore the knowledge of the causative agents of wound infection will be helpful in the control of wound infection and selection of empiric antimicrobial therapy as an infection control measure.

Aim of the study

The purpose of this study is to determine bacterial profile and drug resistance pattern of pathogen isolated from wound at ARTH, Addis Ababa, Ethiopia

Benefits for participants

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

Risks and complication

There are no anticipated risks to your participation. From your wound site swab will be taken once. During collection of pus you may feel some discomfort but this does not produce serious pain.

Confidentiality

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

Assurance of Principal Investigator

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

Sosina Ayalew (PI)

Signature: _____ Date: _____

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

PI Address: Sosina Ayalew : Department of Medical Laboratory Sciences, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: absosina2011@gmail.com Tel.- 0912166324

II. Informed consent

I have been informed about the objective of the study entitled "Bacterial profile, antimicrobial susceptibility pattern and associated risk factor of wound infection at ARTH Addis Ababa, Ethiopia." I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

Therefore, with full understanding of the situations I agree to give the entire necessary information and wound swab for laboratory analysis. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also told that results for the wound analysis was given to the health facility and that I may ask the information if I want.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code:_____ Signature: _____

Date: _____

Annex 3: Amharic Version of the participant information sheet and Consent

ሀ.የተሳታፊዎች የመረጃ ቅፅ

አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስ ዲፓርትመንት

<u>አርስት፡-</u> በቁስል ውስጥ የሚገኙ የባክቴሪያ አይነቶች፣ የፀረ ባክቴሪያ መዳኒት የመቋቋም ባህሪያቸው፣ በጦር ኃይሎች ሆስፒታል አዲስ አበባ ኢትዮጵያ

<u>አጠቃላይ መረጃ፡-</u> በጥናቱ በመሳተፍዎ ከልብ እያመሰገንን ከመወሰንዎ በፊት ይህን ቅፅ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያዳምጡ፤ እንዲሁም ግልጽ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ

<u>ስለጥናቱ መረጃ፡-</u> የቁስል ህመም በህመምተኛው ላይ የተለያዩ ችግሮችን ሊያስከትል ይችላል። ለምሳሌ የህክምና ወጭን ይጨምራል፣ ለህመምተኛው ምቾት ይነሳል፣ ሆስፒታል ውስጥ ተጥቶ የመታከም ጊዜን ያራዝማል ብሎም ለሞት ሊዳርግ ይችላል። ስለዚህም የቁስልን ህመም ሊያመጡ የሚችሉ ባክቴሪያዎችና አባባሽ ነገሮችን ማወቅ ህመሙን ለመቆጣጠርና ለመከላከል ይጠቅማል።

<u>የጥናቱ አላማ፡-</u> በቁስል ውስጥ የሚገኙ የባክቴሪያ አይነቶችን እና የፀረ ባክቴሪያ መድሃኒት የመቋቋም አቅማቸውን ማወቅና ማጥናት ነው።

<u>ጥናቱ ለተሳታፊዎች ያለው ጥቅም፡-</u> በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም፣ ነገር ግን በምርመራው ውጤት መሰረት የመታከም እድል ይኖሮታል። በተጨማሪም የጥናቱ ውጤት የቁስልን ህመም ለመቆጣጠርና ለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ እንዲሁም ህብረተሰቡን የመጥቀም እድል ያገኛሉ።

<u>በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር</u>

በዚህ ጥናት በመሳተፍ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም ለዚህ ጥናት የሚያገለግል ናሙና ከቁስሎ ላይ የሚወሰድ ሲሆን ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም።

<u>የመረጃ ሚስጥራዊ አጠባበቅ</u>

የሚሰጡት መረጃ ወቅትም ሆነ ከዛ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል። በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አልዎት። የላብራቶሪ ውጤትዎን ማወቅ ከፈለጉ የመለያ ቁጥሮን በመጠቀም በሚሰጣቸው የቀጠሮ ጊዜ መውሰድ ይችላሉ።

<u>የጥናቱን የሚካሄደው ሰው ማረጋገጫ</u>

ለዚህ ጥናት ሃላፊነቱን ለመውሰድና፣ ማናቻውንም ጥናቱ የሚመለከቱ ጉዳይ ክትትል ለማድረግና ለሚመለከተው አካል መግለጫ ለመስጠት በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ ----------ቀን ----------

ማንኛውም ጥያቄ መጠየቅ ለሚሹ የሚቀጥለውን አድራሻዬን መጠቀም ይችላሉ፡፡

ኢሜል absasina20011@gmail.com **ስልክ** 0912 16 63 24

II.የፈቃደኝነት ማረጋገጫ ቅጽ

በቁስል ውስጥ የሚገኙ የባክቴሪያ አይነቶችና የፀረ ባክቴሪያ መድሃኒት የመቋቋም አቅማቸው እንዲሁም የቁስል ህመም የሚያባብሱ ነገሮች በሚል ርእስ ላይ ለማጥናት በተመለከተ በሚደረገው ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ አላማና ጥቅም ተገልያልኛል፡፡ በመጠይቁ ላይ የሚመጣው የኑ ሙሉ መረጃም በሚስጥር እንደሚያዝ ተነግሮል፡፡

በተጨማሪም ጥናቱ ውስጥ አለመሳተፍ መብቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡

ስለሆነም ሁኔታውን በሚገባ በማጤን በፍቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪው ፍቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠው የቁስል ናሙና ለተጠቀሰው ጥናት ብቻ እንደሚውል ተነግሮት ተስማምቻለው፡፡ ማንኛውም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ውጤቶች በጊዜው ክትትል ለሚያደርግልኝ የጤና ባለሙያ እንደሚሰጡ እና ውጤቱን ማወቅ ከፈለጉ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ ---------- የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምሩ ላይ ስለእኔ መረጃና የቁስል ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ ----------ቀን ----------

Annex 4: Laboratory data collection form

1. Patient identification

Ward ----------

Age (years) _____

Gender Male ….. Female…….

II. Laboratory Data

1.1Date of specimen collection_____

2..Pus consistency: 1)Clear 2)Yellowish 3)Greenish 4)Bloody 5)Others: Specify_____

3..Media used _____

4..Gram stains result _____

5..Biochemical test_____

6..Organism isolated _____

7.. Drug susceptibility pattern

7.1sensitive to _____

7.2Intermediate to _____

7.3 Resistance to _____

III. Comments_____

Name of principal investigator _____

Signature _____ Date _____

Annex 5: Declaration

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

Name: Sosina Ayalew

Signature _____

Place _____

Date of submission _____

This thesis has been submitted with my approval as University advisor:

Name Gebru Mulugeta (MSc)

Signature _____

Place _____

Date of submission _____