ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES FROM WOUND INFECTIONS AT ALL AFRICA LEPROSY, TUBERCULOSIS AND REHABILITATION TRAINING CENTER, ADDIS ABABA ETHIOPIA.

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A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CLINICAL LABORATORY SCIENCES, DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY SPECIALITY TRACK

June, 2017

ADDIS ABABA, ETHIOPIA

Addis Ababa University

School of graduate studies, Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Asdesach Tessema entitled: **Antimicrobial Susceptibility Pattern Of bacterial isolates From Wound Infections At ALERT Center, Addis Ababa Ethiopia** and submitted in fulfillment of the requirements for the Degree In Master of Science (Clinical Laboratory Science) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

By: Asdesach Tessema (BSc.)

Signed by the examining committee

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Acknowledgment

First of all I would like to thank Almighty God who gave me strength and courage to finish this study. My Sincere gratitude and appreciations goes to my advisors Dr Adane Bitew (PhD, Associate professor) and Mrs. Tsehaynesh Lema (Msc, PhD Fellow) for their unreserved support and professional guidance for the successful accomplishment of this research thesis. I am grateful for AAU, Department of Medical Laboratory Science for giving me an opportunity to conduct this research.

My heartfelt thank and respect goes to all ALERT Center Clinical Laboratory staffs (my work mates) for their unreserved support and friendly co-operation.

My special appreciation goes to staffs in ALERT Center Surgical OPD who supported me during sample collection. I am also grateful for all study participants for their willingness to participate in this study.

I am pleased to thank my family and friends who have been with me throughout my journey.

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List of Abbreviations

AAU	Addis Ababa University
ABR	Antibiotic Resistance
ALERT	All Africa Leprosy, Tuberculosis and Rehabilitation Training Center
AMR	Anti Microbial Resistance
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
CDC	Center for Disease Control and Prevention
CHS	College of Health Science
CLS	Clinical Laboratory Science
CLSI	Clinical and Laboratory Standard Institute
CONS	Coagulase-negative Staphylococci
IDSR	Integrated Disease Surveillance and Response
MDR	Multi Drug Resistance
MIC	Minimum inhibitory concentration
SMLS	School of Medical Laboratory Science
SOPs	Standard operating procedures
SPSS	Statistical Package for Social Science
WHO	World Health Organization

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Abstract

Background: Wound develops into an infected state when the balance between microorganism and the host shifts in favour of the micro-organism. Antimicrobial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs.

Objective: The main objective of this study was to isolate etiology of wound infections and determine their antimicrobial susceptibility pattern.

Methods: A cross-sectional study was conducted at ALERT Center from February to May 2017. Swabs from different types of wounds was taken and processed to isolate etiologic agents by using standard microbiological techniques. Antimicrobial susceptibility tests were performed by disc diffusion technique as per the standard modified Kirby-Bauer method.

Results*:* In this study 171 bacterial isolates were recovered from 188 specimens showing an isolation rate of 86.2%. The predominant bacteria isolated from the infected wounds were *Staphylococcus aureus* 96 (51.1%) followed by *Klebsiella pneumoniae* 26 (15.2%), *Escherichia coli* 23(13.4%). Out of 162 positive samples 9(5.5%) were mixed infections. *Staphylococcus aureus* exhibited highest sensitivity against Clindamycin (95.8%), Gentamycin (94.8%), Chloramphenicol (92.7%), Ciprofloxacillin (89.6%) and Cotrimoxazole (84%). Gram negative isolates, *E.coli, P.vulgaris, P.mirabilis, P.aeroginosa* and *Citrobacter* showed the highest sensitivity against Amikacin (100 %). *E.coli* showed high resistance for Ampicilin (95.7%) and Augumentin (91.3%) where as *P.vulgaris* showed 100% resistance for Ampicilin and 90.9 % for Tetracycline.

Conclusion: There was high prevalence of bacterial isolates in this study. *S. aureus* was the predominant isolate 96 (56.1%). Most of the isolates showed high resistance to commonly used antimicrobials. The antimicrobial profile of drugs demonstrated that the commonly prescribed drugs against Gram positive bacteria (Penicillin, Tetracycline) and Gram-negative bacteria (Ampicillin and Tetracycline) as a single agent for empirical treatment of wound infections would not cover the majority of wounds infections. Antimicrobial treatment should be based on the result of culture and sensitivity.

Keyword: wound infection, bacterial isolates, drug resistance pattern.

1. Introduction

1.1 Background

Antimicrobial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance. Bacterial susceptibility to antibacterial agents is achieved by determining the minimum inhibitory concentration (MIC) and disc diffusion technique that inhibits the growth of bacteria (1).

Bacteria can acquire antibiotic resistance either by mutation or through exchange of genetic material among same or closely related species. The sudden acquisition of resistance to antibiotics poses difficulties in treating infections. Resistance to several different antibiotics at the same time is even more a significant problem. It is because of the acquired resistance that bacterial isolates must be subjected to antibiotic susceptibility testing. Bacteria showing reduced susceptibility or resistance to an antibiotic imply that it should not be used on the patient (1, 2).

The probability of wound infections largely depends on the patients' systemic host defenses, local wound conditions and microbial burden. Wound develops into an infected state when the balance between microorganism and the host shifts in favour of the micro-organism. The conditions of antimicrobial therapy, both prophylactically and therapeutically, can only be defined when these factors are under control (2, 3).

Hence, an ongoing surveillance could play a significant role in the early recognition of a problem and, there is a need for early intervention for better management of wound infections. Exposure of subcutaneous tissue following a loss of skin integrity (i.e. wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Since wound colonization is most frequently poly-microbial, involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected (3).

The antibiotic resistance to microbes leads to severe consequences. Infections caused by resistant microbes fail to respond to treatment resulting in prolonged illness and greater risk of death, longer periods of hospitalization and infections which increases the number of infected people moving in the community. When an infection becomes resistant to first line antibiotic, treatment

has to be switched to second or third line drugs, which are always much more expensive and sometime more toxic as well (1).

In poor countries, where many of the second or third line therapies for drug resistant infections are not available, making the potential of resistance to first line antibiotics considerably greater. The limited number of antibiotics in these countries are becoming increasingly inadequate for treating infections and necessary antibiotics to deal with infections caused by resistant pathogens are absent from essential drug list (1, 3).

Skin and soft tissue infections that usually follow minor traumatic events or surgical procedures are caused by a wide spectrum of bacteria. Involvement of antibiotic resistant organisms in these infections, increase the difficulty of their treatment and may have significant influence on the ultimate outcome. Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogens, an understanding of the pathophysiology of the infectious process and of the pharmacology and pharmacokinetics of the intended therapeutic agents (4, 5).

Despite the progress made with respect to infection control and wound management, wound infection still remains a serious and significant clinical challenge particularly in developing countries (6). This is because; wound site infections are a major source of post-operative illness, a cause of death among burn patients, and accounts for approximately a quarter of all nosocomial infections (5). To this effect, isolation and characterization of bacteria implicated in causing wound infections and determining drug susceptibility pattern of the etiologic agents, for efficient management of patients with wound infections is still an active field of research. Although a number of studies have been conducted on wound infections in Ethiopia, a shift in etiologic agents and poor laboratory setup coupled with development of drug resistance warranted additional investigation. Against this background, the aim of this study was to identify and determine drug susceptibility pattern of bacteria isolated in wound infections from patients at ALERT Center.

1.2. Statement of the Problem

Antibiotic resistance is now a major issue confronting healthcare providers and patients. Changing antibiotic resistance patterns, rising antibiotic costs and the introduction of new antibiotics have made selecting optimal antibiotic regimens more difficult now than ever before. Furthermore, history has taught us that if we do not use antibiotics carefully, they will lose their efficacy (7).

Evidence from around the world indicates an overall decline in the total stock of antibiotic effectiveness: resistance to all first-line and last-resort antibiotics is rising. The patterns of which bacteria are resistant to specific antibiotics differ. The U.S. Centers for Disease Control and Prevention (CDC) estimates that antibiotic resistance is responsible for more than 2 million infections and 23,000 deaths each year in the United States, at a direct cost of $20 billion and additional productivity losses of $35 billion. In Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections, costing €1.5 billion annually in direct and indirect costs (8, 9).

Until the 1970s, many new antibacterial drugs were developed to which most common pathogens were initially fully susceptible, but the last completely new classes of antibacterial drugs were discovered during the 1980s. It is essential to preserve the efficacy of existing drugs through measures to minimize the development and spread of resistance to them, while efforts to develop new treatment options proceed (10).

Information concerning the true extent of the problem of antimicrobial resistance (AMR) in the African region is limited. There is a scarcity of accurate and reliable data on Antimicrobial resistance (AMR) in general, and on Antibiotic resistance (ABR) in particular, for many common and serious infectious conditions that are important for public health in the region. The World Health Organization (WHO) Member States endorsed the Integrated Disease Surveillance and Response (IDSR) strategy in 1998. Effective implementation of IDSR is a way to strengthen networks of public health laboratories, and thus contribute to effective monitoring of AMR. However, a recent external quality assessment of public health laboratories in Africa revealed weakness in antimicrobial susceptibility testing in many countries (8, 10).

According to a team led by World Health Organization (WHO) researchers report developing countries had much higher infection rates than the developed world and it is said “poor nation face: greater hospital infection burden”. Wound infection results from microbes thriving in the surgical site because of poor preoperative preparation, wound contamination, improper antibiotic selection, and the lack of ability of an immunocompromised patient to fight against infection (10, 11).

Use of antibacterial drugs has become widespread over several decades and these drugs have been extensively misused by humans and that favor the selection and spread of resistant bacteria. Consequently, antibacterial drugs have become less effective or even ineffective, resulting in an accelerating global health security emergency that is rapidly outpacing available treatment options. A shift in etiologic agents and poor laboratory setup coupled with development of drug resistance warranted additional investigation in developing countries. Various studies have been done on the prevalence and antimicrobial resistance patterns of wound infections in Ethiopia. These studies indicated that high prevalence of bacterial isolates and many of the bacterial isolates showed high levels of resistance to most commonly prescribed drugs like, amoxicillin, tetracycline, chloramphenicol, erythromycin while low levels of resistance to gentamicin, cloxacillin, norfloxacine and ciprofloxacin were documented (5, 7, 11).

1.3. Significance of the Study

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goal of the test is to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen antimicrobial agents, or to detect resistance in individual bacterial isolates.

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.

In Ethiopia, drug resistance pattern is highly increasing from time to time according to various studies due to misuses of antibiotic by public. Hence this study is very essential to see the pattern of resistance and the result of this study will assist clinicians to prescribe the appropriate antibiotics and helps the patients in getting timely and appropriate treatment.

2. Literature review

The prevalent organisms that have been associated with wound infection include *Staphylococcus aureus* (*S. aureus*) which from various studies have been found to account for 20-40% and *Pseudomonas aeruginosa* (*P. aeruginosa*) 5-15% of the nosocomial infection (5).

A study was conducted in Pattukkottai, Tamilnadu, India in 2013 to assess Antibiotic susceptibility of bacterial strains isolated from wound infection. A total of seventy wound swab specimens were collected and cultured of which all samples showed bacterial growth. Six different species of bacteria were isolated. *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%). Majority of the bacterial isolates were resistant to almost all the antimicrobials employed. Among all the bacterial isolates, *Pseudomonas aeruginosa, E.coli and Klebsiella pneumonia*e 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 bacteria recovered (13).

Similar retrospective study was conducted in Amravati city, India to determine Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antibiotic agents. A total of 78 bacterial isolates were recovered from 258 specimens showing an isolation rate of 31.2%. The predominant bacteria isolated bacteria in their study were *Staphylococci* 36 (46.2%), followed by *Streptococci* 18 (23.1%) Gram negative *Pseudomonas aeruginosa* 12(15.4 %) and *proteus* spp 8 (10.4%). The Gram positive and Gram negative bacteria constituted 68 (87.2%) and 10 (12.8%) of bacterial isolates; respectively Gram positive microorganisms were sensitive to chloramphenicol 44.4%, azithromycin 22%, cefotaxime 22%, amoxiclav 11.1%, ciprofloxacin 11.1%.Gram negative *E.coli* were sensitive to erythromycin,ciprofloxacin, chloramphenicol. *Pseudomonas* was sensitive to levofloxacin, azithromycin, ofloxacin, tetracycline, imipenem, sparfloxacin and amoxiclav. (14).

In Nepal another study was carried out to assess antimicrobial Susceptibility Patterns of the Bacterial isolates in Post-operative wound infections. 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 (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% (16).

One Nigerian study was conducted in one of Nigerian town, Kano to determine Incidence and Antibiotic Susceptibility Pattern of Bacterial Isolates from Wound Infections. Out of the 150 specimens collected, 82 % were infected with bacteria made up predominant of *S. aureus* (22 %), *P. aeruginosa* (19.9 %), *Citrobacter* spp (15 %), *E. coli* (14.7 %) and *P. mirabilis* (14.5 %). Antibiotic susceptibility tests showed that *P. aeruginosa* was susceptible to ceftazidime, ciprofloxacin and gentamicin while the enteric bacteria resistance to ceftazidime, gentamicin and ciprofloxacin (17, 18).

A cross-sectional study was conducted in South India to assess Aerobic Bacterial Profile and Antimicrobial Susceptibility Pattern of Pus Isolates. Out of 114 pus samples received for culture and sensitivity, 102 (89.47%) cases yielded positive culture while 12 (10.53%) cases had no aerobic growth. Among the 102 culture positive pus samples, 97 showed pure bacterial isolates and 5 yielded mixed growth; so a total number of 107 organisms were isolated out of 102 pus samples. *Staphylococcus aureus* was the most common isolates followed by *P. aeruginosa* , *E. coli , K. pneumoniae , S. pyogenes* , *S. epidermidis* and *Proteus* spp .Among the Gram positive isolates , vancomycin , levofloxacin and clindamycin were the most susceptible drugs whereas among the Gram negative isolates, the most susceptible drugs were piperacillin / tazobactum , levofloxacin , imipenem and amikacin (19).

Isolation and identification of different bacteria from different types of burn wound infections and their antimicrobial sensitivity pattern was carried out in Primeasia University, Banani, Dhaka, Bangladesh. Out of 150 wound swabs 100 samples were found positive. *P. aeruginosa* was found to be the most common isolate (23.33%) followed by *S. aureus* (15.33%), *Enterobacter* spp. (8.66%), *P. vulgaris* (8%), *Micrococcus* spp. (3.33%), *E. coli* (4.66%) and *Klebsiella* spp. (3.33%). Among eight antibiotics, Ciprofloxacin was found to be the most

effective drug against most of the Gram-negative and Gram-positive isolates followed by Amikacin, while Chloramphenicol, Doxycycline and Gentamicin were less sensitive (20, 21).

Similar Retrospective Chart Review study was conducted by Muhammad Naveed Shahzad *et al*. to determine bacterial Profile of burn wound infections in burn patients. Their finding showed that single isolates were present in 57.85 % of cases and multiple isolates were noted in 34.65 % cases. The frequency of Gram negative organisms was high. The most common isolate was *Pseudomonas aeruginosa* 54.4%, followed by *Staphylococcus aureus* 22.00%, *Klebsiella* spp. 8.88%, *Acinetobacter* spps-4.63%, *S. epidermidis* 5.79 %, *Proteus* spp 2.70% and *E. coli* 1.54% (22).

A cross-sectional descriptive study was done on 1150 hospitalized neonates in neonatal intensive care unit (NICU) wards of Ecbatana hospital of the Hamadan University of Medical Sciences from September 2004 to September 2006 to assess Antibiotic sensitivity pattern of common bacterial pathogens. The cultures were positive in 105 cases (25.2%). 60 male neonates (57.1%) and 45 female neonates (42.9%) were culture positive. The most common microorganisms isolated were *E. coli* 66.7% (70 cases), *Klebsiella* spp. 10.5% (11 cases). Drug resistance was high in these microorganisms (23).

A study done in India from February to April 2014. Out of 63 samples, 42 bacterial isolates of 6 species were isolated which included 2 species of Gram positive bacteria and 4 species of Gram negative. 21-30 age groups were found to be the most vulnerable age group in both males and females. *S. aureus* was found to be most predominant followed by *S. epidermidis*. The most sensitive antibiotic was Vancomycin (100%) while the least effective antibiotic was Amoxicillin (35%) followed by Penicillin (36%). Their study revealed that the bacterial pathogens isolated showed resistance to most of the antibiotics:-Penicillin, Ampicillin, Ciprofloxacin, Ofloxacin, Gentamycin (19, 23).

A study which was conducted in North East Ethiopia focused on bacteriology and antibiogram of pathogens from wound infections. Analyzed 599 wound swab samples. Out of which 422 (70.5%) were culture positive. 78(18.5%) of the culture had double infections. *S.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 Coagulase negative *Staphylococcus* (1.8%). Amoxicillin had the highest resistance rate of 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 and cloxacillin (7).

A cross-sectional study was conducted in Jimma University Specialized Hospital, South-West Ethiopia to determine, antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents. In that study 145 bacterial isolates were recovered 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* spp. 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. The overall multiple drug resistance patterns were found to be 85%. They have concluded that on *in-vitro* sensitivity testing, ampicillin, penicillin, cephalothin and tetracycline were the least effective whereas- gentamicin, ciprofloxacin, vancomycin and amikacin were the most effective antibiotics (5).

A retrospective study was conducted in Gondar Teaching Hospital, to assess patterns and multiple drug resistance of bacteria pathogens isolated from wound infection. Bacterial pathogens were isolated from 79 patients showing an isolation rate of 52%. *S.aureus (65%)* was the predominant species followed by *E.coli* (10%), *Klebsiella pneumoniae* 9%, *Proteus* spp. 4% and *Streptococci* spp. 4%. Among 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%), and tetracycline and co-trimoxazole (63%). The overall multidrug resistance pattern was 78.5% (26, 27).

3. Hypothesis

The Antimicrobial susceptibility pattern of bacterial isolates from wound infections in ALERT Centre was the same with previous similar study conducted in Jimma, Ethiopia.

4. Objectives of the study

4.1. General objective

- To determine the Antimicrobial susceptibility pattern of bacterial isolates from wound infections at ALERT Center Addis Ababa Ethiopia.

4.2. Specific objectives

- To isolate and identify common bacterial pathogens that cause wound infections.
- To determine antimicrobial susceptibility pattern of bacterial isolates

5. Materials and Methods

5.1. Study Area

The study was conducted in patients with only wound infections attending ALERT Centre, Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia, with a population of 3,384,569 according to the 2007 population census conducted by the Central Statistical Agency of Ethiopia (CSA, 2007) with annual growth rate of 3.8%. Based on this estimation, the population in the year 2015 would be 4,478,127. Addis Ababa lies at an altitude of 2324 m (7625 ft.) above sea level and located at 8°58'N, 38°47'E and has a mean annual temperature and rainfall of 15.9 $^{O}_{C}$ and 1089 mm, respectively. ALERT Center is one of the specialized tertiary referral hospitals in the country. It is located in Addis Ababa at 7 kms south west on the way to Jimma. ALERT main mission was to provide training for both genders in multiple aspects of Leprosy including prevention, treatment and rehabilitation in an African context. Its main mission was based on provision of quality service and training for Leprosy, Rehabilitation, Surgery, dermatology, Ophthalmology and relevant infectious diseases. Currently it has widened its scope and has opened a surgical outpatient department (OPD) at which patients with any wound are managed and treated, daily in average 50 patients visit this department. This shows that there is high burden of wound infection at ALERT Center.

5.2. Study design and Study period

A cross sectional study was conducted from February to May 2017 at ALERT Center.

5.3. Population

5.3.1. Source population

All patients with any wound infections who visited ALERT Center during the study period.

5.3.2. Study population

All patients with any wound infection who visited ALERT Center Surgical Outpatient Department (OPD) during the study period that fulfills the eligibility criteria.

5.4. Sample size determination

The sample size was calculated based on single sample size estimation. The value of p taken as 87.3% (0.873) from the previous study conducted on Antimicrobial susceptibility pattern of

bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia (5). Considering 95% confidence interval, 5% margin of error and 87.3 proportions, the sample size was calculated using the following standard formula. Contingency for the unknown circumstance was taken as 10%.

$$N = \underline{(Z\alpha/2)^2 * (1-p) * (p)} / (d)^2$$

Where n=sample size estimated

α = level of significance

z = at 95% confidence interval Z value (α = 0.05) =>Zα/2=1.96

d= Expected margin of error =0.05

p=prevalence of previous study found from literature review= 87.3% (4)

$n = (1.96)^2\ 0\ .873(1\text{-}0.873)/(0.05)^2$

n=168 + 10% unknown circumstance

n=185

5.5. Sampling Technique

A consecutive sampling technique was used. All consecutive patients who came to ALERT Center Surgical OPD with wound infection were included.

5.6. Selection and evaluation of study subjects

Convenient sampling technique was applied to select the study subjects. Thus, a careful clinical examination of patients was conducted by physicians assigned. All Patients with wound infection that fulfill the eligibility criteria during the study period were selected.

5.7. Inclusion and Exclusion criteria

5.6.1. Inclusion criteria

- Patients with any open wound infection
- Patients that agreed to participate in the study and give informed consent.

5.6.2. Exclusion criteria

- ❖ Patients who were on antibiotic treatment within 15 days of data collection.

5.8. Study variables

5.8.1. Dependent variables

- Antimicrobial susceptibility pattern
- Bacterial isolates

5.8.2. Independent variables

- Age
- Sex
- Occupation
- Educational background

5.9. Data collection procedures

Structured and Predesigned questionnaire was developed and used for collection of data on socio-demographic characteristics (age, sex, occupation and educational back ground of patients).

5.9.1. Sample collection, handling and transport

Open wound swabs were aseptically obtained after the wound immediate surface exudates and contaminants was cleansed off with moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed off 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 within 30 minutes.

5.9.2. Sample analysis

Culture, Gram staining and Biochemical tests

Swabs collected from patients were streaked on a blood agar (5% sheep blood) and MaCconkey agar (Oxoid) by sterile inoculating loop. The plates were incubated at 35–37°C for 24–48 hours. Preliminary identification of bacteria was done based on colony characteristics of the organisms. Some colony characteristics like haemolysis on blood agar, changes in physical appearance in

differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from pure cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests-Oxoid using: - Kliger Iron Agar (KIA), Indole test, Simmon's citrate agar, Lysine Iron Agar (LIA), urea and motility. Gram-positive cocci were identified based on their Gram-reaction, catalase and coagulase test results. Mannitol salt agar was used also as a differential media to differentiate coagulase positive from coagulase negative *Stapylococci* (CoNS).

Antimicrobial susceptibility testing (AST)

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique (27) according to criteria set by Clinical and Laboratory Standard Institute (CLSI) 2016. The inoculum was prepared from pure culture by picking parts (3-5) of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. The test organism was uniformly seeded over on Mueller-Hinton agar (Oxoid) surface and exposed to antibiotic diffusing from antibiotic impregnated paper disks into the agar medium, and then incubated aerobically at 37°C for 16–18 hours. Diameters of zone of inhibition around the discs were measured to the nearest millimeter using a clipper and classified as sensitive, intermediate, and resistance according to the standardized table supplied by CLSI 2016.

Only the conventional antibiotics regularly available for frequent use in the study area was considered for this study and all the disks that are used for the test were from Oxoid. The following antimicrobial agents were employed:- Penicillin (10iu) Ceftriaxone (30µg), Clindamycine (10µg), Erythromycin (15µg), Gentamycin (10µg), Ciprofloxacin (5µg), Tetracycline (30µg), Ampicillin (10µg), Augumentin (30 µg), Amikacin (30µg), Cefepime (30µg), Cotrimoxazole (25µg), Chloramphenicol (30µg) and Ceftazidime (30µg).

5.10. Quality Control

All specimens were collected by following standard operating procedures (SOPs). The sterility of culture media was checked by incubating 5 % of each batch of the prepared media at 35-37 ºC for 24 hours. Performance of catalase reagent (3% hydrogen peroxide) was checked by known *S. aureus* (positive control) and *S. pyogenes* (negative control). Coagulase test was checked by known *S. aureus* (positive control) and *S. epidermidis* (negative control). For oxidase test *P.*

aeruginosa (positive control) and *Escherichia coli* (negative control) was used. Any physical changes like cracks, excess moisture, color, hemolysis, dehydration & contamination were checked before use of all culture medias. Also expiration date was checked strictly. The qualities of all reagents were checked. Temperature of incubator and refrigerator was monitored daily. All prepared media were checked by inoculating standard strains, such as *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC-25922) and *P. aeruginosa* (ATCC-27853) from ALERT Center Microbiology Laboratory as a quality control during study period for culture, Gram stain and antimicrobial susceptibility testing.

5.11. Data Management

All data obtained from patients/guardians was kept on a secured, password protected computer. Hard copies of the data collection sheets were kept securely locked and archived to protect clients confidentiality.

5.12. Data Analysis

Data entry and analysis was performed by using SPSS statistical software version 20. The descriptive statistics was calculated for each variable using frequencies and crosstabs.

5.13. Ethical Consideration

Ethical approval was obtained from Department Ethics and Research Committee (DERC) of AAU, (COHS), SOHS, department of Medical Laboratory Sciences. Permission was also obtained from ALERT Center for data collection. Written informed consents were obtained from each individual after the purpose of the study explained. For children, consent was obtained from the parent or guardian of the child. The purpose of the study was explained to the participants and also they have been informed about their right to refuse or to participate in the study and the confidentiality of the information gathered. The study participants with positive results were referred to the physician who examined them for the prescription of appropriate drugs based on drug sensitivity testing.

5.14. Operational Definitions

Wound:- Exposure of subcutaneous tissue to bacterial infection following a loss of skin integrity (29).

Resistance: - A category that implies that an isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely (e.g. beta-lactamases), and clinical efficacy has not been reliable in treatment studies (30).

Intermediate: - A category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used (30)

Susceptible*:* - A category that implies an infection due to the isolate may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated (30).

MDR:- A term that describe the organism resist for two or more drugs of different groups (11)

6. Result

6.1. Socio demographic characteristics

A total of 188 study participants were enrolled in this study. Among these, 72 (38.3%) were females and 116 (61.7%) males. The ages of the participants ranged from 1 year to 83 years with mean age of 31.8 ± 17.02 (Table1). In this study, wounds were collected from different body sites (Table 2). Most of the causes of the wound were identified (Table 3). Wound infection was the highest 120 (68.3%) in patients of age group 15-44 followed by 32 (17.0%) age groups of 45-64. Age was classified based on Provisional Guidelines on Standard International age classification (31).

Table 1. Socio demographic characteristics of study participants at ALERT Center from February to May, 2017.

Variables	Characteristics	Frequency (%)
Sex	Male	116 (61.7)
	Female	72 (38.3)
Age	≤14	24 (12.8)
	15-44	120 (68.3)
	45-64	32 (17.0)
	≥65	11 (5.9)
Level of education	Illiterate	41 (21.8)
	Elementary	65(34.6)
	High school	42(22.3)
	College and above	32(17.0)
	Under age	8(4.3)
Occupation	Government employee	21(11.2)
	Private enterprise	33(17.6)
	Day laborer	28(14.9)
	Merchant	4(2.1)
	House wife	22(11.7)
	Farmer	7(3.7)
	No job	26(13.8)
	Under age	17(9.0)

Table 2. Location of wound from patients at ALERT Center from February to May, 2017.

Wound site	Frequency (%)
Leg	67(35.6)
Foot	39(20.7)
Arm	26(13.8)
Finger	18(9.6)
Face	13(6.9)
Scalp	12(6.4)
Back	10(5.4)
Chest and abdomen	3(1.6)
Total	188 (100%)

Table 3. Causes of wounds from infected patients at ALERT Center from February to May, 2017.

Causes	Frequency (%)
Accident	92 (48.9)
Operation (Surgical)	46 (24.5)
Unknown causes	26 (13.8)
Burn	17 (9.1)
Animal bites	7 (3.7)
Total	188 (100)

6.2. Isolated bacterial profile

Out of the 188 swabs taken 162 (86.2%) were culture positive for bacterial pathogens, while 26 (13.8%) culture showed no growth. Out of 162 positive samples 9(5.5%) were mixed infections and a total of 171 bacterial isolates were identified. Among the isolates, *Staphylococcus aureus* 96 (56.1%) was the predominant. *Klebsiella pneumoniae* 26 (13.8%) was the most frequently isolated Gram negative bacteria followed by *E. coli* 23 (12.2%). *Pseudomonas aeruginosa* (3.5%) and only one *Citrobacter* spp. (0.6%) was isolated. The proportion of each bacterial isolate to the total isolates is presented in Table 4.

Table 4.Magnitude of bacterial isolates from wound infection at ALERT Center from February to May, 2017.

Bacterial isolates	Frequency	Percentage
Staphylococcus aureus	96	56.1
Klebsiella pneumonia	26	15.2
Escherichi coli	23	13.4
Proteus vulgaris	11	6.4
Proteus mirabilis	8	4.7
Pseudomonas aeruginosa	6	3.5
Citrobacter spp.	1	0.6
Total	171	100

In our study, we found mixed infections. A total of 9 mixed bacteria were isolated. Percentage frequency of mixed bacterial isolates from wound infection is presented in Table 5.

Table 5.Magnitude of mixed bacterial isolates from wound infections at ALERT Center from February to May, 2017.

Bacterial Isolates	Frequency	Percentage
S.aureus and *E.coli*	3	33.333
S.aureus and *K.pneumoniae*	3	33.333
E.coli and *P. vulgaris*	3	33.333
Total	9	100

6.3. Antimicrobial susceptibility patterns of different bacterial isolates

Antibiotic susceptibility of the isolated organisms was determined by standard Kirby-Bauer disk diffusion method. *Staphylococcus aureus* exhibited highest sensitivity against Clindamycin (95.8%), Gentamycin (94.8%), Chloramphenicol (92.7%), Ciprofloxacillin (89.6%) and Cotrimoxazole (84%). In this study *S.aureus* showed resistance for Penicillin (66.7%) and Tetracycline (46%) only.

Among Gram negative isolates, *E.coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa* and *Citrobacter* showed the highest sensitivity against Amikacin (100 %) and for *K.pneumoniae* Amikacin showed 96.2% sensitivity. *E.coli* showed high resistance for Ampicilin

(95.7%) and Augumentin (91.3%) where as *P.vulgaris* showed 100% resistance for Ampicilin and 90.9 % for Tetracycline (Table 6).

Table 6.Antimicrobial susceptibility pattern of bacterial isolates from wound infections at ALERT Center from February to May, 2017.

Isolated Organisms	**AST**	**Antibiotics**												
		P	E	DA	CIP	GEN	CHL	COT	TE	AMK	CRO	CAZ	CFP	AMP	AUG
S.aurues (n=96)	S	26.0	67.7	95.8	89.6	94.8	92.7	84.0	45.8	-	-	-	-	-	-
	I	7.3	6.3	1.1	2.1	1.0	1.0	0	7.3	-	-	-	-	-	-
	R	66.7	26.0	3.1	8.3	4.2	6.3	16.0	46.9	-	-	-	-	-	-
E.coli (n=23)	S				73.9	69.6	73.9	43.5	8.7	100	65.2	78.3	78.3	0	4.3
	I				4.4	4.3	0	0	8.7	0	0	4.3	0	4.3	4.3
	R				21.7	26.1	26.1	56.5	86	0	34.8	17.4	21.7	95.7	91.3
P.vulgaris (n=11)	S				72.7	63.6	45.5	54.5	9.1	100	30.0	70.0	90.0	0	20.0
	I				-	9.1	10.0	0	0	0	0	0	0	0	30.0
	R				27.3	27.3	45.5	45.5	90.9	0	70.0	30.0	10.0	100	50.0
P.mirabilis (n=8)	S				62.5	62.5	25.0	25.0	0	100	50.0	62.5	62.5	12.5	25.0
	I				0	0	0	12.5	25.0	0	0	0	0	0	25.0
	R				37.5	37.5	75.0	62.5	75.0	0	50.0	37.5	37.5	87.5	50.0
K.pneumoniae	S				77.0	65.4	57.7	57.7	19.2	96.2	61.5	73.1	73.1	7.7	11.5
	I				3.8	7.7	7.7	3.8	27.0	3.8	0	0	0	0	84.7
	R				19.2	26.9	34.6	38.5	53.8	0	38.5	26.9	26.9	92.3	3.8
P.aeruginosa (6)	S				83.3	83.3	-	-	-	100	-	83.3	100	-	-
	I				16.7	0	-	-	-	0	-	0	-	-	-
	R				0	16.7	-	-	-	0	-	16.7	-	-	-
Citrobacter spp. (n=1)	S				0	0	0	0	0	100	0	0	0	0	0
	I				0	0	0	0	0	0	0	0	100	0	0
	R				100	100	100	100	100	0	100	100	0	100	100

Key:- P=Penicillin, E=Erythromycin, DA=Clindamycin, CIP=Ciprofloxacillin, GEN=Gentamycin, CHL=Chloramphenicol, COT=Cotrimoxazole, TE=Tetracycline, AMK=Amikacin, CRO=Ceftraxione, CAZ=Ceftazidime, CFP=Cefepime, AMP=Ampicillin, AUG=Augumentin, S=Sensitive, I=Intermediate, R=Resistance AST= Antimicrobial susceptibility testing

Higher rate of MDR (100%) was seen among *Citrobacter* spp., *P. mirabilis*, and *E. coli* but lower rate of (20%) MDR isolates seen among *P.aeruginosa*

Table 7. Antibiogram of bacteria isolated from patients with infected wounds at ALERT Center from February to May, 2017

	No. (%) of resistance							
Bacterial isolates	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6-10**	**MDR**
Staphylococcus aureus (n=96)	15(15.6)	34(35.4)	25(26.0)	12(12.5)	3(3.1)	4(4.2)	3 (1.6)	47(83.9)
Klebsiella pneumoniae (n=26)	-	3 (11.5)	5 (19.2)	5 (19.2)	-	3(11.5)	10(38.5)	23(88.4)
Escherichi coli(n=23)	-	-	3 (13.0)	7 (30.4)	3(13.0)	3 (13.0	7 (30.4)	23(100)
Proteus vulgaris (11)		1(9.1)	2(18.2)	1(9.1)	2(8.2)	-	5(45.5)	10(90.9)
Proteus mirabilis(n=8)	-	-	1(12.5)	1(12.5)	2(25.0)	-	4(50.0)	8(100)
Pseudomonas aeruginosa (6)	5(83.3)	-	1(16.7)	-	-	-	-	1(20)
Citrobacter spp. (n=1)	-	-	-	-	-	-	1(100)	1(100)
Total	20(7.3)	38(22)	37(13.6)	26(9.5)	10(5.8)	10(5.8)	30(17.5)	**113(66.1)**

Key R0 = no resistance to antibiotic ,R1= resistance to 1 antibiotics. R2= resistance to 2 antibiotics R3=resistance to 3 antibiotics, R4 =resistance to 4 antibiotics, R5 =resistance to 5antibiotic , R 6-10=resistance to 6-10 antibiotics

7. Discussion

Of the 188 clinical samples collected from patients with cases of wound infections, bacteria have been identified in 162 patients giving a isolation rate of 86.2%.Though the prevalence rate of wound infections in the present study was within the reported range, it was relatively the same prevalence rates of 87.3%, and 70.5% reported in similar studies conducted in South west and North East Ethiopia; respectively (5, 7). This study also has similar prevalence rate with studies conducted in Nepal, Nigeria and India in the rate of 80%, 82% and 89.5 % respectively (15, 16 and 18). Both local and abroad studies showed similar bacterial isolates in the range of 70.5% to 89.5%, this shows similarity may be due to following Standard operating procedures strictly for bacteria isolation.

The type and the relative frequencies of bacteria causing wound infections vary greatly among studies. In the present study, among 171 bacterial isolates, 96 (56.1%) were Gram-positive, i.e *S.aureus* and 75(43.9%) were Gram-negative. Among Gram negative bacterial isolated, *K. pneumoniae* was found in 26 patients (15.2%), *E. coli* in 23 (13.4%) and *P. vulgaris* 11(6.4%) patients. In this study, *S. aureus* and *K. pneumoniae were* the major bacteria associated with wound infection. The same have been reported by Araya G., *et al.,* (32), Esebelalie *et al*., (33). *E. coli* as a third predominant isolate following *S. aureus* and *K. pneumoniae* has been documented by Mama M., et al and Shriyan *et al* (5, 4).

The same study conducted in Ethiopia, had shown *E. coli* as the first most prevalent. However in our study *K. pneumoniae* 26 (15.2%) was the predominant Gram negative bacteria. Variation in the distribution of microbial agents between different geographical locations and regions within the same country may be responsible for this diversity. The possible reason for the high frequency of *Staphylococcus aureus* is that this bacteria commonly found in human skin as normal flora and wherever it gets breaks on skins and soft tissue they can easily disseminate besides cross contamination of wound from nasal colonization by *S. aureus* could be one possible explanation for high isolation rate of *S. aureus,* illustrating the importance of preventing cross-contamination in hospital environments as it was explained by Onwubiko *et al.,* (17). In our study *P. aeruginosa* was among the least isolated bacteria and this might be due to only 17 (9.0%) of wound swabs were taken from burn patients whereas; it was highly prevalent in most other studies (7, 21 and 34).

The prevalence rate of mixed infections (5.3%) observed in this study was lower than 34.6% reported in previous study by Anil *et al.,* (21). This may be due to the difference in identification methods that is known to influence the relative prevalence of bacteria which makes comparison of results difficult.

Based on CLSI guideline 2016 we have used selected drugs for *Pseudomonas aeruginosa* which were available in the study area during study period. Among drugs guided bay CLSI 2016, we have utilized Gentamycin, Ciprofloxacillin, Ceftazidime, Amikacine and Cefepime. In present study *P. aeroginosa* showed high sensitivity for most drugs, 100% for Amikacin and Cefepime, 83.3% for Gentamycin, Ciprofloxacillin and Ceftazidime. There was no resistance bacterium isolated in our study for selected drugs. The relatively low level of resistance to these drugs may be, these drugs had been in the market for a relatively low availability most of the time as compared to drugs such as tetracycline, ampecilin and erythromycin. Our result was similar with study conducted in Jima, Ethiopia by Mama *et al* (5) but not concurrent with results documented in Nepal by Anil *et al* and Salu Rai (20, 38), this might be due to variation in geographical location and drug consumption trend.

Given that the majority of therapy for wound infections is empiric and that bacteria associated with wound infections are demonstrating increasing anti-microbial resistance, continuously updated data on antimicrobial susceptibility patterns would be beneficial to guide empiric treatment. In our study, both Gram-positive and Gram-negative bacteria were tested for drug susceptibility against a panel of eight drugs for Gram positive and eleven drugs for Gram negative bacteria. The number of drugs tested against bacteria isolated from wound infections in the present study was more or less the same number and family of drugs tested in previous studies in Ethiopia (5, 7, 34, 35). This may play vital role to identify if there is a shift in a drug resistance pattern for the similar drugs used in previous studies.

The overall drug resistance rates of Gram-negative bacteria isolates ranged from 3.8% for Augumentin and 10% for Cefepime to 100% for Ampicillin, and 90% for Tetracycline. This figure demonstrates that Ampicillin and Tetracycline as a single agent for empirical treatment of wound infections would not cover the majority of wounds infected by Gram negative bacteria in the study area. High level of drug resistance to Ampicillin and Tetracycline in the present study was compatible with results of similar studies conducted locally (5, 7, 35, 36) and from abroad

(21, 23 and 24). Availability of these anti-microbial agents without prescription and inappropriate dosing schedules may explain the isolation of high level of drug resistance against these drugs and other drugs such as Penicillin.

S. aureus showed an average resistance rate of 22.2% to most of the antimicrobial drugs tested which is relatively similar with previous studies done locally by Mama *et al* and Mulugeta *et al* (5, 7). In present study *S. aureus* showed highly resistance for Penicillin 66.7% and this is concurrent with study conducted locally by Hailu *et al* (33), but lower than study conducted by Mama *et al* (5). Study conducted in Nigeria by Onwubiko N. *et al* (17), the resistance rate of Penicillin for *S. aureus* was very low, i.e. 7.1 % only. From various drugs used in our study for *S. aureus*, Clindamycin (95.8%), Gentamycin (94.8%), Chloramphenicol (92.7%) and Ciprofloxacillin (89.6%) showed high sensitivity. This finding has an agreement with study conducted locally in Jimma and Dessie by Mama et al and Mulugeta *et al* (5, 7). It has also shown an agreement with study conducted in Karnataka by Kaup *et al* (6). In the current study, Tetracycline (49.6%) showed slightly high resistance which was lower than the study conducted by Mulugeta K *et al.,* (7) but it was similar with the study conducted in Karnataka by Kaua *et al* (6).

K. pneumoniae, the first most common Gram-negative bacterium isolate was sensitive to Amikacin (96.2%) and Ciprofloxacillin (77.0%) and was intermediate for Augumentin (84.7%). The average resistance rate for this isolate was 44.2% and it was comparable with the results documented from previous studies by Derese *et al* and Zarrin *et al.,* (36, 37).

The second most common Gram-negative isolate in our study was *E .coli* 23 (13.4%). It was highly sensitive for Amikacin (100%) and this result is the same with the study conducted by Mama *et al* (5) and showed low resistance to Gentamycin (3.2%), Ciprofloxacillin (2.7%), Cefepime (2.7%), Ceftazidime (2.1%), and Ceftraxione (4.3%). High resistance was observed for Ampecillin (95.7%), Augumentin(91.3%) and Tetracycline (86%). This resistance rate observed in our study was similar with study conducted in Southwest- Ethiopia and South India (5, 37).

P. vulgaris and *P. mirabilis* showed high sensitivity for Amikacin (100%). Both isolates showed sensitivity for Cefepime, 90% and 62.5% respectively. *P. vulgaris* showed high resistance for Ampeciline 100% and Tetracycline (90%) whereas *P. mirabilis* showed a resistance rate of

87.5% and 75% for Ampicillin and Tetracycline respectively. These results were comparable with various studies conducted in Addis Ababa, Jimma, Mekele, India (35, 5, 13 and 19).

We have isolated a single bacterium of *Citrobacter* spp. in our study and it was 100% resistance for all drugs tested except Amikacin which is 100% sensitive and was 100% intermediate for Cefepime.

Over all MDR rate of isolated bacteria in this study was 66.1%. This finding was similar with MDR rate reported by Mulugeta *et al* (7) but lower than 95.5%, 85% , 82.1% resistance rate reported by Mulu *et al*., Mama *et al* and Sewunet *et al* respectively (27, 5, 34) and Mohammad *et al* in Nepal (16).

8. Limitations

It was not possible to include obligate anaerobic bacteria due to poor laboratory facilities in the study area.

We couldn`t utilize much drugs as we planned due to drugs unavailability on market.

9. Conclusion

The results of this study illustrated a prevalence rate of wound infection and drug susceptibility pattern of bacteria isolated from wound infection. Out of the 188 swabs taken 162 (86.2%) were culture positive for bacterial pathogens. Of 162 positive samples 9(5.5%) were mixed infections and a total of 171 bacterial isolates were identified. Among the isolates, *Staphylococcus aureus* 96 (56.1%) was the predominant. *Klebsiella pneumoniae* 26 (13.8%) was the most frequently isolated Gram negative bacteria followed by *E. coli* 23 (12.2%). High prevalence rates of wound infection necessitate a continuous epidemiological survey of wound infection in health institutions across the country.

Staphylococcus aureus exhibited highest sensitivity against Clindamycin (95.8%), Gentamycin (94.8%), Chloramphenicol (92.7%), Ciprofloxacillin (89.6%) and Cotrimoxazole (84%).

Gram negative isolates, *E.coli, P.vulgaris, P.mirabilis, P.aeruginosa* and *Citrobacter* showed the highest sensitivity against Amikacin (100 %). *E.coli* showed high resistance for Ampicilin (95.7%) and Augumentin (91.3%) where as *P.vulgaris* showed 100% resistance for Ampicilin and 90.9 % for Tetracycline. The antimicrobial profile of drugs demonstrated that the commonly prescribed drugs against Gram positive bacteria (Penicillin, Tetracycline) and Gram-negative bacteria (Ampicillin and Tetracycline) as a single agent for empirical treatment of wound infections would not cover the majority of wounds infections. Replacement of these drugs with drugs that are more potent and appropriate based on drug sensitivity testing is needed.

10. Recommendations

- Continuous surveillance to monitor etiology and antimicrobial susceptibility patterns both in the community and hospital settings is needed to guide the empirical use of antimicrobials
- National surveillance of antibiotic resistant organisms and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaigns is necessary.
- Other etiologic agents of wound infection, like anaerobic bacteria, fungus and other micro-organisms that can be important causes of infections shall be done in the future.
- Antimicrobial treatment should be based on the result of culture and sensitivity to minimize drug resistance pattern.

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Annex 1. Information sheet for the study participants

Date.....

Greetings!

Introduction

Hello, how are you?

My name is …… and I am MSc student of Addis Ababa University, School of Medical laboratory Sciences. I am doing a research entitled "Antimicrobial Susceptibility Pattern of Bacterial Isolates from wound infection in ALERT centre, Addis Ababa Ethiopia".

Purpose of the study

The objective of this study is to determine Antimicrobial Susceptibility Pattern of Bacterial Isolates from wound infection.

Duration: The duration of this study depend upon the availability of study subjects. It might take about three months or more.

Risk associated with the specimen collection: The risk associated with the specimen collection is minimal since the collection of these specimens will follow the routine procedures for the laboratory investigation. There might be a little discomfort during sample collection.

Procedure of the study

If you agree to participate in the study, sample will be collected from the wound with moistened sterile swab by principal investigator.

Confidentiality

All the data obtained will be kept strictly confidential and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

Benefit

There will not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. The result will be given to you and if your result is clinically significant, it will help you for further diagnosis and treatment.

Withdrawal rights

Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are free

to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits to which you are entitled such as proper care and treatment. Your access to treatment will not be dependent on your participation in the study.

If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation to a great extent.

-If you have any question regarding to this study, the address of the principal investigator is:

Principal Investigator: **Asdesach Tessema**
Tel: +251-912100231
Email: asdesacht@gmail.com or asdesacht@yahoo.com

DMLT, AAU

Tel: +251-112755170

Annex 2: Information sheet for the study participants in Amharic

አጠቃላይ መረጃ

አዲስአበባ ዩንቨርሲቲ የድህረምረቃ ት/ትቤት

የላብራቶሪ ሳይንስ ትምህርት ክፍል

በጥናቱየሚሳተፉግለሰቦችየፈቃድመጠየቂያእናመቀበያፎርም/ሺት/

መግቢያ፡

ሰላም እንደምን አሉ?

ስሜ----------እባላለሁ:: የኢ.አ.ዩ. የላበራቶሪ ሳይንስ ትምህርት ክፍል የማስተርስ ዲግሪ ተማሪ ነኝ በአሁኑ ሰአት የቁስል ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን የስርጭት መጠን በአለርት ማእከል ለማወቅ ጥናት እያካሄድኩ ነው፡፡

የጥናቱ ዋና አላማ፡

የጥናቱ አላማ የቁስል ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት ቁስል ህመማን ላይ ምን ያህል እንደሆነ ለማውቅ ነው፡፡

የጥናቱ ጊዜ፡ ከትትል በሚያደርጉ ቁስል ታካሚዎች ብዛት የሚወሰን ሲሆን 3 ወር እና ከዛም በላይ ለወስድ ይችላል፡፡

ሊከሰቱ ስለሚችሉ ስጋቶችና የምችት መጓደሎች፡ለጥናቱ በሚወሰደዉ ናሙና ምክንያት የተለየ ችግር አይከሰትም፡፡ የሚያሰጋ ምንም ነገር የለዉም ምክንያቱም የጥናቱ ናሙና አወሳሰድ ከወትሮዉ በሸተኛዉ ለራሱ ብሎ ከሚሰጠዉ የተለየ አይደለም፡፡ ናሙና በሚወሰድበት ሂደት ከትንሽ የህመም ስሜት ውጪ ይሄ ነው የሚባል ችግር የሚያስከትል ወይም የሚያሰጋ አይደለም፡፡

የጥናቱ ሂደት፡

እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ ከቁስሎት ላይ ጥናት ለሚያከዉኑ ባለሙያ ናሙና ይሰጣሉ፡፡

የጥናቱ ሚስጢራዊነቱ፡

የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀነው፡፡በስም አይጻፉም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል፡፡ ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስምም ወይም ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም፡፡ ከምርመራ የሚገኘውም ውጤት ወይም ሌላ መረጃ ለሚመለከታቸው አካላት ለምሳሌ፤ እርስዎን የሚንከባከቡ የህክምና ባለሙዎች እና ጥናቱን ለሚያካሄዱት ባለሙያዎች እንዲሁም ጥናቱ ስነምግባርን ጠብቆ መከናወኑን ለሚከተሉት የኮሚቴ አባላት ብቻ ይገለፃል፡፡ ኮምፒውተር ላይ ያሉ መርጃዎች ምስጢራዊነታቸው የተጠበቀ ሲሆን በወረቀት ያሉ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃሉ፡፡

የሚሰገኘው ጥቅም፡

በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም ነገር ግን የአይን ኢንፌክሽን ተህዋስያን ህመም ካለዉ ወይም የምርመራ ውጤቱ ህክምና የሚያስፈልገው ከሆነ ተጨማሪ ምርመራ እና ህክምና እንዲያገኙ የረዳዎታል፡፡ ስለሆነም ከጥናቱ በሚገኘው እውቀት ቁስል ኢንፌክሽን ተህዋስያን ባክቴሪያ አማካኝነት የሚመጣውን በሽታ በተሻለ ደረጃ ለመቆጣጠርና ለበሽታው ትክክለኛውን ፀረ ባክቴሪያ ለመምረጥ ሀኪሞችን ይረዳል፡፡

ከጥናቱ ስለማቋረጥ፡

በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኃላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡ በጥናቱ መሳተፍ ወይም አለመሳተፍ አገልግልት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም፡፡ ጊዜዎትን መስዕዋት አድርገው ስለተባበሩኝ ከልብ አመሰግናለሁ፡፡

ስለ ጥናቱ ሕጋዊነት ለመጠየቅ ከፈለጉ፡

ይህንን ጥናት አስመልክቶ ጥያቄ ካለዎት ወይም የጥናቱ የመጨረሻ ዉጤት ምን እንደሆነ ለማዉቅ ከፈለጉ በሚከተለዉ አድራሻ ሊያገኙን ይችላሉ፡፡

የጥናቱአስከያጅ፡ _አስደሳች ተሰማ

ሰ.ቁ፡-0912100231

ኢ.ሜል: Email: asdesacht@gmail.com or asdesacht@yahoo.com

DMLT, AAU

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Annex 3. English version of the questionnaire

The title of this study is **"**Antimicrobial susceptibility pattern of bacterial isolates from wound infections" attending ALERT centre, Addis Ababa, Ethiopia

Interview

We are grateful for your agreement to participate in this study. Now we are going to have an interview with you and the interview is about general socio demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore we respectfully ask you to give us the right response.

A.	**Background information**	
1	Study ID	
2	Participant Card No.	
3	Address	Rural ☐ Urban ☐ Region:_____KefleKetema:_____Kebele:_____ Tele phone: _____
4	Full name of the Participant:	
5	Sex	Male ☐ Female ☐
6	Age:	
7	Occupation?	Student ☐ Daily laborer ☐ Government employee ☐ Merchant ☐ Private enterprise employee ☐ Housewife ☐ No job ☐ Under age ☐ Farmer ☐
8	Level of education?	Illiterate ☐ Elementary ☐ High school ☐ College and above ☐ Under age ☐

	What is the cause of the wound?	Surgical ☐ Burns ☐ Bites (insect, animal or snake) ☐ accident ☐ Others (specify)…… ……..
9	Sites of the wound	
	Have you been sick of this disease for last months of time?	No ☐ Yes ☐
10	Do you take a medication to treat these infections?	No ☐ Yes ☐ if yes when?_____ Type of medicine you take?_____
11	Date of specimen taken and time?	
B. comments		

Other information

1. Media used _____
2. Organisms isolated _____
3. Drug susceptibility pattern
   - 3.1. Sensitive to ----------
   - 3.2. Resistance to----------
   - 3.3. Intermediate to----------
4. Biochemical tests for gram positive bacterial isolates
   - 4.1. Coagulase----------DNAse---------- Catalase----------
5. Biochemical tests for gram negative bacterial isolate
   - 5.1. Indole _____ citrate agar_____ KIA_____ lysine decarboxylase agar_____ urea agar _____motility medium_____ Manitol_____
6. Gram reaction result from culture ----------

Other remarks ----------

Annex 4. Consent form

For adult patients who are able to respond:

I have been requested to participate in this study, which plans to determine Antimicrobial Susceptibility Pattern of Bacterial Isolates from wound infection in ALERT centre, Addis Ababa Ethiopia in which I will be benefited from study. I have been informed this study which involves collecting swab from wound. During collection of the specimen I have been told that there is no harm except little discomfort and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care and hospital access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use the specimen taken from wound for the investigation. Moreover I have had the opportunity to ask questions about the project and I have received clarification to my satisfaction. I was also told that results will be reported timely to the requesting physicians for the appropriate treatment and management of the wound infection.

I agree that I am contributing to the treatment of my fellows by participating in this project. I have asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I_____ hereby to approve my agreement with my signature.

I _____, after being fully informed about the detail of this study, hereby give my consent to participate in this study, if the participants are volunteer.

_____	_____	____/____/___
Name of adult patients	Signature	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year

Annex 5. Consent form in Amharic

የስስምምነት መጠየቂያ ቅጽ

በዚህ ጥናት ለሚዳሰሱ ጥናቱች ሀሳባቸዉን መግለጽ ለሚችሉ

እኔ----------በአለርት ማዕከል የቁስል ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በቁስል ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ የተዘጋጀ ጥናት ላይ እድሳተፍ ተጠይቄ ስለጉዳዩም ለመረዳት በቂ መረጃ አግኝቻለሁ፡፡ ስለሆነም ናሙና የሚሰበሰበው ከቁስል መሆኑን ስለተርዳሁኝ ናሙና ወስዶ መመርመር አስፈላጊ ስለሆነ ናሙናዉን በመስጠት ልተባበር ሙሉ ፈቃደኛ መሆኔን ገልጫለሁ፡፡ ናሙና በሚወስድበት ወቅት ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት እንደሌለው ተነግሮኛል እንዲሁም ከመጠይቁ አንብቢያለሁ ወይም ተነቦልኛል፡፡ ከምርመሩ መሳተፍ ወይም አለመሳተፍ መብቴ የተጠበቀ መሆኑን እና ላለመሳተፍ ብወስን በአለርት ሆስፒታል በሚደረግልኝ ህክምና ላይ ምንም ተፅዕኖ እንደማይኖረዉ ተረድቻለሁ፡፡

ስለዚህ የጥናቱን ጠቃሚነት አምኜበት የስምምነት ቃሌን የሰጠሁት በፍፁም ፈቃደኝነት ነዉ፡፡ በመጨሻም እኔ ከጥናቱ ዉጤት ተጠቃሚ ልሆን እንደሚችል ተገልፆልኝ በመሳተፌና በመተባበሬ ወገኖቼን ልረዳ በመቻሌ ደስተኛ መሆኔን ገልጨ፤ ግለፅ ያልሆኑ ጥያቄዎች ላይ ማብራርያ እንዲሰጠኝ ጠይቄ መልስ ተሰጥቶኛል፡፡ እንዲሁም በጥናቱ ሂደት እንድሳተፍ ፍቃደኝነቴን በፊርማዬ አረጋግጠለሁ፡፡

_____	_____	_____/_____/____
የተሳታፊው ሥም	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	የምስክር ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	__/__/__
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

Annex 6. Parental consent form in English

I_____ parent, after being fully informed about the purpose of this study, Study title: **"**Antimicrobial susceptibility pattern of bacterial isolates from wound infections**"** at ALERT centre, Addis Ababa, Ethiopia

I, the undersigned, have been told about this research. My child has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

_____	_____	____/____/___
Name of adult parent	signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)		Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

Annex 7. Parental consent form in Amharic
የስምምነት መጠየቂያ ቅጽ

እኔ----------የልጄ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በአለርት ማዕከል በቁስል ታካሚዎች መሀከል የቁስል ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በቁስል ታማሚዎች ላይ ምን ያህል እንደሆነ ለማውቅ በጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ልጄ ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የልጄ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____/_____/____
የተሳታፊው ሥም	ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	የምስክር ፊርማ	ቀን /ወር/ዓ.ም
___ _____	_____	_____/_____/____
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

Annex 8. Guardian consent form in English

I_____ guardian, after being fully informed about the purpose of this study, Study title: **"**Antimicrobial susceptibility pattern of bacterial isolates from wound infection**"** at ALERT centre, Addis Ababa, Ethiopia.

I, the undersigned, have been told about this research. My guardian has to say to choose if I want to be in the study. I have been informed there is no harm except little discomfort during sample collections. I have been informed that other people will not know my guardian results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my guardian. By signing below I agree to let my guardian to participate in this research study.

_____	_____	____/____/___
Name of guardian	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)		Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

Annex 9. Guardian parental consent form in Amharic

የስስምምነት መጠየቂያ ቅጽ

እኔ----------የታማሚው አሳዳጊ/ሞግዚት ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በአለርት ማዕከል በተመላላሽ የቁስል ታካሚዎች መሀከል የቁስል ኢንፌክሽን ተህዋስያን የሚያመጣውን ህመም እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት በቁስል ህሙማን ላይ ምን ያህል እንደሆነ ለማውቅ በጥናቱ ታማሚው እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም ስሜት ውጪ ምንም አይነት ጉዳት ታማሚው ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም ታማሚው መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ታማሚው በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጇ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ ታማሚው ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር ታማሚው በጥናቱ ውስጥ ለመካተት የእኔ አሳዳጊ/ሞግዚት ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ታማሚው በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____/_____/____
የጥናቱ ተሳታፊ የአሳዳጊ/ሞግዚት ስም	የጥናቱ ተሳታፊ ፊርማ	ቀን / ወር/ ዓ.ም
_____	_____	_____/_____/____
ምስክር (ማንበብና መፃፍ ለማይችሉ)	የምስክር ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/____
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

Annex 10: Assent form for adolescent (12 -17 years old) study participants (English version)

Study title: **"**Antimicrobial susceptibility pattern of bacterial isolates from wound infections**"** at ALERT centre, Addis Ababa, Ethiopia.

I, the undersigned, have been told about this research. My parents or guardian have to say to choose if I want to be in the study. I have been informed there is there is no harm except little discomfort during sample collections. I have been informed that other people will not know my results as it coded with number rather than writing my name if I am in this study. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my parents/guardian. By signing below I agree to participate in this research study.

_____	_____	____/____/___
Name of Adolescent	Signature	Day/month/year
_____	_____	____/____/___
Witness (Illiterate)	Signature	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature	Day/month/year

Annex 11: Assent form for adolescent (12-17 years old) study participants (Amharic version)

በአማርኛ የተዘጋጀ ዕድሜያቸዉ ከ12 እስከ 17ዓመት ለሆኑታዳጊ ወጣት የጥናት ተሳታፊዎች የተሳትፎ ማራጋጋጫቅጽ

ከዚህ በታች ስሜ የተገለፀው በዚህ ጥናት ውስጥ እንድሳተፍ ፍቃደኝነቴን ተጠይቂያለሁ፡፡ ወላጆቼም/ አሳዳጊዎቼም በጥናቱ እንድሳተፍ ወይም እንዳልሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና ሲወሰድ ከትንሽ የህመም የህመም ስሜት ዉጪ ምንም አይነት ጉዳት እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የእኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ በግሌ የማገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በእኔ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የኔ በጥናቱ ውስጥ ለመካተት የወላጆችም ወይም የአሳዳጊዎቸ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በፍቃደኝነቴ በጥናቱ እንደምሳተፍም ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____/_____/___
የጥናቱ ተሳታፊ ስም	የጥናቱ ተሳታፊ ፊርማ	ቀን / ወር/ ዓ.ም
_ _____	_____	_____/_____/___
ምስክር (ማንበብና መፃፍ ለማይችሉ)	የምስክር ፊርማ	ቀን /ወር/ዓ.ም
_____	_____	_____/_____/___
የተመራማሪው ስም	ፊርማ	ቀን /ወር/ዓ.ም

Annex 12: 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 ^{0}C in a carbon dioxide atmosphere (candle jar) and the MacConkey agar plate aerobically.

8. Examine and report the culture; if the cultures 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 thei r 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 will be 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 will be 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 colonies of test organisms 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 or physiological saline.

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 inoculums 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”

Declaration

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