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Addis Ababa University

This is to testify that the thesis prepared by Getachew Seyoum which is entitled with "*Multidrug resistant bacteria in Postoperative Wound infection at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia*" And submitted in partial fulfillment of the requirements for the degree of Master of Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

MULTIDRUG RESISTANT BACTERIA IN POSTOPERATIVE WOUND INFECTION AT TIKUR ANBESSA SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA.

Approved by the Examining Board

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List of abbreviations

AST----------Antimicrobial Susceptibility Test

CDC---------- Center for Disease control

CLSI----------Clinical and Laboratory Standards Institute

CONS----------*Coagulase Negative Staphylococci*

EMA----------Ethiopian Medical Association

EMLA ----------Ethiopian Medical Laboratory Association

EPHA ----------Ethiopian Public Health Association

HIV ----------Human Immunodeficiency Virus

NNIS----------National Nosocomial Infections Surveillance

QC----------Quality control

SOPs ----------Standard Operating Procedures

SPSS ----------Statistical Package for the Social Sciences

SSI----------Surgical site infection

WHO ----------World Health Organization

Abstract

Background: Post operative wound infection is hospital acquired infection and it is the major cause of morbidity and mortality throughout the world especially in developing countries. Drug resistance is the challenge for controlling such bacteria now days. Assessing the prevalent bacteria and testing their antibiotic susceptibility helps to provide effective therapies, develop rational prescription writing and make policy decisions.

Objective: To determine the prevalence of multidrug resistant bacteria in postoperative wound infection.

Method: A cross sectional study was conducted from March 30 to August 28/2015 at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. A total of 197 study participants who had been diagnosed for post operative wound infection during the study period were included. All collected pus samples were cultured on Blood, MacConkey and Mannitol salt agar. All culture positive samples were characterized by gram stain and standard biochemical tests. Bacterial antimicrobial susceptibility test was performed on Muller-Hinton agar using Kirby-Bauer method.

Results: The overall prevalence was 75.6% (n=149/197) and the predominant bacteria isolated were *S. aureus33.3%* (n=56/168), *E. coli 14.3% (n=24/168 and Coagulase negative Staphylococci* 11.3% (n=19/168). Double infections 11.4% (n=17/149) were seen in which *S. aureus* and *Pseudomonas species* showed common association 23.5% (n=4/17). Multidrug resistances were recorded in 65.5% (n=110/168) of all bacterial isolates. Gram positive and Gram negative isolates showed 55.3% (n=42/76) and 73.9% (n=68/92) multiple drug resistance respectively. Among the antibiotics tested amoxicillin (93.5%), Ceftriaxone (85.3%), Penicillin (84.5%) and Cefotaxime (82.7%) showed high level of resistance. Clindamycin (7.9%) and Amikacin (1.1%) showed high sensitivity for Gram Positive and Gram negative respectively

Conclusion: The choice of drugs in the treatment of bacterial isolates from postoperative wound infections is quite narrow today due to the wide scale resistance of pathogens to common drugs. Rational use of antibiotics and a regular monitoring of antimicrobial resistance patterns in post-operative wound infections are essential and mandatory to prevent further emergence and spread of anti-microbial resistance among bacterial pathogens.

Key terms: postoperative wound infections**,** Multidrug resistance bacteria, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

1 Introduction

1.1 Background

Postoperative wound infection(Surgical site infections (SSIs)) are defined as infections that occur within 30 days of the operation if no implant is left in place or within 1 year of operation if an implant is left in place and the infection appears to be related to the operation [1]. These infections are further defined by their anatomic location superficial infections (47%)involve only skin or subcutaneous tissue of the incision; deep infections (23%)involve the fascia and muscle layers; and organ space infections (30%)involve any part of the anatomy (other than the incision) that was opened or manipulated during the operation[2]. The infection follows interference with the skin barrier and it is associated with the intensity of bacterial contamination of the wound at surgery or later in wards during wound care [3].

Postoperative wound infections are the third most commonly reported nosocomial infections. And it accounts for approximately a quarter of all nosocomial infections [4]. It has been responsible for the increasing cost, morbidity and mortality related to surgical operations and continues to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative propitiation and antibiotic prophylaxis [1, 4]. Patients who develop postoperative wound infections are 60% more likely to spend time in an intensive care unit, are 5 times more likely to be readmitted to the hospital and are twice more likely to die than patients without these infections [2, 5].

For years wounds have been classified in to four categories according to the theoretical number of bacteria that contaminate wounds: clean, clean-contaminated, contaminated and dirty[5, 6]. Wound infection rates in large series are approximately1.5 to 3.9 percent for clean wounds, 3 to 4 percent for clean-contaminated wounds and approximately 8.5 percent for contaminated wounds. Dirty wounds are generally left open, but wound infection rates for 12 dirty wounds of 28 to 40 percent have been reported [6]

Bacterial pathogens that able to survive in the hospital environment for long period and resist disinfection are particularly more important for nosocomial infections [7]. *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species*, *Proteus species*,

Citrobacter species and Coagulase negative staphylococci are the most notorious nosocomial pathogens [8, 9]. Their ability to survive in the hospital environment by developing resistance to antimicrobial/disinfectant agents makes them the main criminal as aetiological agents in nosocomial surgical wound infections. Their capabilities to colonise rapidly in a compromised host make them very difficult to deal with. These infections caused by nosocomial pathogens need special attention, if uncontrolled, may become life-threatening. The important reason for this is their resistance to many currently available antibiotics [10, 11].

The source of postoperative wound infections can be either endogenous or exogenous [12]. Patients may be infected by their body flora following surgical manipulation which in most cases suppresses the natural body defense mechanisms [12, 13] and exogenous sources of infections include hospital staff, other patients and visitors, food, water, fomites [14, 15]. Each hospital has its own unique bacterial flora to which patients are at risk for acquiring infection during hospitalization [1]. Surgeons employ a logical approach towards postoperative wound infection control only when such epidemiologic data are available and also resistance to antimicrobials has become a serious problem necessitating the in-depth study of postoperative wound Infection to prevent the future complications in operated cases[1, 16].

1.2 Statement of the problem

As indicated above postoperative wound infections are the third most commonly reported nosocomial infection and have been responsible for the increasing cost, morbidity and mortality related to surgical operations and continues to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative propitiation and antibiotic prophylaxis [4]. More importantly unrestrained and rapidly spreading antimicrobial resistance among bacterial populations has made the management and treatment of postoperative wound infections a serious challenge in clinical and surgical practice [19]. Hence patients with post-operative wound infections face additional exposure to microbial populations circulating in a hospital set up as the hospital environment is always charged with microbial pathogens. Most postoperative wound infections are hospital acquired, and vary from one hospital to the other and are associated with complications, increased morbidity and mortality [20].

Despite the advance in modern medicine, nosocomial infection still poses a risk of increased morbidity and mortality to patients [21]. Now days the emergence of poly antimicrobial resistant strains of hospital pathogens has also presented a challenge in the provision of good quality inpatient care. The battle between bacteria and their susceptibility to drugs is yet problematic among public, researchers, clinicians and drug companies who are looking for effective drugs [22]. In addition to this, postoperative wound infection by resistant bacteria worsens the condition and it has become serious problem in developing countries owing to poor infection prevention program, crowding hospital environment and irrational prescription of antimicrobial agents [23].

Common bacteria which are associated with postoperative wound infections are *Staphylococci aureus, Klebsiella species, Escherichia coli, Proteus species, Streptococcus species, Enterobacter species, Pseudomonas species and Coagulase negative Staphylococci* [17, 18]. In recent years, drug resistant bacteria have given rise to several serious outbreaks of infections with many deaths [11].

Postoperative wound infections are the most frequent types of nosocomial infections in developing countries and the rate of these infections are markedly higher in many developing countries [3]. These infections would worsen patient management when they caused by

multidrug resistant bacteria [16]. In Ethiopia postoperative wound infections are the most common hospital acquired infections where rational use of drugs is poorly practiced and weak trend of strict adherence to postoperative wound infection prevention guide lines [25].

In Ethiopia some studies have been conducted on postoperative wound infections [21, 22, 23, 25, 26]. However these studies are insufficient as compared to the rate of postoperative wound infections and occurrence of multidrug resistant bacteria. In addition these studies showed that the increasing prevalence of postoperative wound infections and high multidrug resistance patterns of bacteria isolates suggesting further studies to be conducted in different parts of Ethiopia. More over antibiotic resistance is changing day by day [11, 16]. Hence the aim of this study is to determine the prevalence of multidrug resistant bacteria in postoperative wound infections at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015

1.3 Significance of the Study

This study provides current information on the prevalence of bacteria that cause postoperative wound infections. Most importantly this study indicates the current picture of antimicrobial resistance patterns on bacterial isolates of postoperative wound infections as antibiotic resistance is changed over time. This will help clinicians to provide safe and effective empirical therapies, develop rational prescription writing, make policy decisions and finally assess the effectiveness of all. In addition this will help for policy makers to amend or develop new infection control programs in local situations.

2 Literature reviews

Nosocomial infection is defined according to the National Nosocomial Infections Surveillance (NNIS) as “a localized or systemic condition that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and that was not present or incubating at the time of admission to the hospital”. The common definition of nosocomial infection is, an infection which occurs within 48 hours after hospitalization, or after 3 days from discharging, or 30 days from an operation [27].

A study conducted in Nepal showed that of the 120 pus swabs processed for culture, 96 showed bacterial growth. *Staphylococcus aureus36* (37.5%) was the predominant gram positive isolate and Escherichia coli24 (25%) was the major gram negative isolate. The infection was most prevalent in the age group 21 - 40 years. 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%. A high level of AMR was observed in gram negative bacterial isolates [28].

Study conducted in Iran by Mohebbi N et al showed the rate of infection after a clean-contaminated surgery was 53%. The most common gram positive microorganism was *Staphylococcus aureus* (22%), and among gram negative: *Escherichia coli(26%), Klebsiella sp(26%) and Pseudomonas species (25%*)[29].

According to the study conducted in India from the 100 patients recruited who underwent surgery in the orthopedic, obstetrics and gynecology and surgical departments Gram positive organisms were more prevalent than gram negative bacteria accounting for 47(67. 14%) and 23(32.85%) of isolates respectively. The three most commonly isolated bacterial species were *S. aureus* 41(58.6%), *Pseudomonas aeroginosa* 10(14. 3%) and *E. coli* 06(8.6%). Out of 41 *Coagulase positive Staphylococcus aureus*, 20 (48.78%) were Methicillin resistant and 21 (51.21%) were methicillin sensitive strains. MRSA were found to be highly resistant to many antibiotics showing only intermediate sensitivity to cefazollin (5 0%) and chloramphenicol(55%)[1].

A study conducted in Turkey showed that *Pseudomonas aeruginosa* was isolated from 16.4 % (152/928) of the patients in ICUs. The highest *Pseudomonas aeruginosa* isolation was obtained in the burns unit (26.9%, 78/290) followed by, cardiovascular surgical ICU (17.6%, 13/74) general surgical ICU (24/164, 14.6 %), internal ICU (17/180, 9.4%) and coronary ICU (20/220, 9.1%). The most effective antibiotics were carbapenems (imipenem and meropenem) and the resistance rates were detected as 15% and 20.4%, respectively among 152 *Pseudomonas aeruginosa* strains [30].

A study conducted in Nigeria which was carried out to determine the prevalence of different pathogens in surgical wounds showed that out of a total of 45 surgical wound specimens analyzed, *Staphylococcus aureus* was isolated from 33(42.30%), *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli* from 25(32.90%), 10(12.80%), and 10(12.80%), respectively. The antibiotic susceptibility of *Staphylococcus aureus* were; ciprofloxacin 60%, erythromycin 40%, gentamycin 60%, streptomycin 60%. Resistance to betalactam antibiotics was common among gram negative bacteria. Some isolates of *Pseudomonas aeroginosa* were resistant to Gentamycin 18.70% and Streptomycin 35.70 %[31].

Another survey was conducted in logos Nigeria, to determine the prevalence of Pseudomonas aeroginosa in Postoperative wound. Swab samples were collected from patients who had undergone operation, sinks, washbasins, floor and nursing staff. Out of the 60 bacterial isolates found in postoperative wound infection, 20 (33.3%) were *Pseudomonas aeroginosa*, followed by *Staphylococcus aureus* 13 (21.7%), *Klebsiella species* 10 (16.7%), *Escherichia coli* 7 (11.7%), Atypical Coliforms 4 (6.7%), *Proteus species* 4 (6.7%), *Streptococcus pyogens* 1 (1.7%) and *Enterococcus faecalis* 1 (1.7%). The in vitro sensitivity pattern of 20 isolates of Pseudomonas aeruginosa showed colistin 100%, gentamicin 75%, streptomycin 30%, and tetracycline10% were sensitive [32].

A cross sectional study conducted in Uganda was on drug sensitivity patterns of bacterial isolates from septic postoperative wounds showed that Pathogenic bacteria were recovered from 58.5% of the specimens and the major isolates were: *S. aureus* 45.1%, Coliforms 16.9%, *Proteus mirabilis* 11.3%, *P.aeruginosa* 9.9%, *Klebsiella pneumoniae* 7.0% and *Enterobacter species* 2.82% [8].

A study conducted in Egypt showed that *P. aeruginosa* and its MDR phenoype accounted for 19 % and 9.5% respectively of nosocomial infections. They detected high rates of MDR *P. aeroginosa* (52%) and of ESβLs producer strains (45.6%) and those ESBLs strains were all MDR. Amikacin and imipenem were the most effective drugs against P. aeruginosa.23 different resistance patterns were identified, profiles from (1 - 8) were prevalent. The most prevalent antibiotype (2) included 12 MDR isolates, 9 clinical and 3 environmental isolates having same patterns. 61.5% of ESβLs isolates harbor plasmids. Each had the same antibiotype and plasmid profile [33].

A study conducted in Ethiopia, Hawasa, indicated that from the 100 samples collected from infected post- operative wounds, 92 (92%) were positive for aerobic culture. The most dominant isolates were *Staphylococcus aureus, Klebsiella spp., Escherichia coli and coagulase negative staphylococci (CoNS*) accounting for 45 (25.4%), 32 (18.1%), 30 (16.9%) and 26 (14.7%) of the isolates respectively. Other bacteria isolated include *Pseudomonas aeruginosa (9.0%), Proteus spp. (6.8%), Streptococci (5.1%), Citrobacter spp. (2.3%) and Enterobacter spp*. (1.7%). Of the 177 isolates, 173 (97.7%) were resistant to at least one antimicrobial, while 164 (92.7%) were resistant to ≥2 antimicrobials. Resistance of isolated organisms was 76.3% to amoxicillin, 71.2% to penicillin, 56.9% to vancomycin, 39.5% to ceftriaxone and Norfloxacin and 31.1% to gentamicin. The susceptibility of S. aureus was 64.4% to gentamicin but it was 100% resistant to amoxicillin. All isolates of *P. aeruginosa* were resistant to penicillin and amoxicillin. The rate of resistance of S. aureus to 2 or more antimicrobials was 97.8% and that of *P. aeruginosa* was 100% [34].

Another study conducted in Ethiopia at Bahir Dar showed that out of 294 patients who had clean and clean-contaminated operation, 10.9% were confirmed of bacterial nosocomial infections. The rate of nosocomial infections among clean and clean-contaminated operations was 3.3% and 12.8% respectively. Nosocomial surgical site and blood stream infection rate was 10.2% and 2.4% correspondingly. A total of 42 bacterial pathogens were identified of which *S. aureus* was the leading isolates accounting26.2% followed by *E. coli* and *CONS* species each 21.4%. Nearly 100% of Gram positive and 95.5% of Gram negative bacterial isolates showed resistance against two or more antimicrobial drugs [35].

A study conducted in Southern part (Jimma) of Ethiopia showed that 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 species 23 (16%), Coagulase negative Staphylococci 21 (14.5%), Klebsiella pneumoniae 14 (10%) and Pseudomonas aeroginosa* 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% [36].

Another study conducted in Gondar showed that 268 bacterial pathogens were recovered from all specimens processed in the study. Most of the isolates, 142 (52.9%) were from the hospital environments such as medical devices, inanimate objects and air. The rest, 77 (28.8%) and 49 (18.3%) were recovered from the health professionals and patients, respectively. predominant organisms associated with postoperative SSIs were *Staphylococcus aureus* 11 (22.4%) *followed by Klebsiella species 10 (20.4%) and Proteus species 9 (18.4%), Escherichia coli6 (12.2%), Enterobacter species and coagulase negative staphylococci each 4 (8.2%), Pseudomonas aeruginosa3 (6.1%) and Citrobacter species* 2 (4.1%)[37].

A study conducted in Debremarkos showed that of the 184 surgical patients who had developed surgical site infection, S. aureus was isolated from 73(39.7%) cases. Out of the 73 isolates of *S. aureus*, 36 (49.7%) were MRSA. Among the study participants, prevalence of MRSA was found to be 19.6%. The clinical isolates showed >80% level of resistance to ampicillin, amoxicillin, penicillin G, erythromycin, gentamicin and cotrimoxazole whereas <50% level of resistance was observed against clindamycin, oxacillin, tetracycline and vancomycin. MRSA strains showed resistance ranging from 5.6%(vancomycin) to 100% (cotrimoxazole)[38].

Another study conducted in Ethiopia, Mekele, showed that out of the 128 wound swabs taken, 96/128 (75%) were culture positive aerobically, yielding 123 bacterial isolates. The predominant bacterial isolates were *Staphylococcus aureus* 44 (35.77%), *Klebsiella species* 29 (22.76%) *and Coagulase negative Staphylococci (CoNS)* 18 (14.63%). No bacterial isolates was found to be sensitive to all antibiotics tested. Isolated bacteria showed 102/123 (82.92%) multi drug resistance to the commonly used antibiotics in the hospital. However, 54/ 65 (83.1%) of Gram negative and 58/58 (100%) of Gram positive isolates were sensitive to Gentamicin and Vancomycin, respectively [39].

Another study also conducted in Ethiopia, at Tikur anbessa specialized hospital , Addis Ababa university showed that, a total of 162 bacteria pathogen were isolated from the open fracture wound sampled. *S.aureus* was the dominant isolate (14.8%), followed by *Acinetobacter spp*. (11.4).The gram positive and gram negative bacteria accounted for 34.0% and 66.0% respectively. All gram-negative bacteria isolate showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and amoxicillin for which they showed (60-80) intermediate level resistance. Fifty-one percent of the gram-negative bacteria isolates were identified as multiple drug resistance [43].

Hypothesis

The overall prevalence of multidrug resistance bacteria from postoperative wound infections is different from the previous.

3 Objectives

3.1 General Objective

- To determine the prevalence of multidrug resistant bacteria in postoperative wound infection.

3.2 Specific Objectives

- To determine the prevalence of bacteria isolates from postoperative wound infections.
- To determine the resistance pattern of bacteria isolates to different commonly used antimicrobial drugs.

4 Materials and Methods

4.1 Study area

The study was conducted at Tikur Anbessa Specialized Hospital which is found in Lideta Sub-city, Addis Ababa Ethiopia. In 1998 it was handed to Addis Ababa University by the Ministry of Health as a teaching hospital for the medical faculty. The faculty is the largest and the oldest among health training institutions in the country, staffed with the most senior specialists and subspecialists. It is now the main teaching hospital for both clinical and preclinical trainings of most disciplines and it is where specialized services are rendered. The Hospital provides tertiary level referral treatment and is known to be open 24 hours for emergency services. The hospital is estimated to also offer diagnosis and treatment for approximately 370,000-400,000 patients yearly with 800 beds, 130 specialists and 50 non teaching doctors.

4.2 Study Design and Study Period

A cross sectional study design conducted from March 30 to August 28/2015

4.3 Source Population

- All patients who were selected for surgical procedures at Tikur Anbessa Specialized Hospital during the study period.

4.4 Study Population

- All patients who develop postoperative wound infection during the study period.

4.5 Eligibilities criteria

4.5.1 Inclusions Criteria

- All patients who gave informed consent and/or assent to participate on the study.
- All surgical patients operated during the study period with presence of post-operative wound infection.

4.5.2 Exclusion Criteria

- Infection occurring within 30 days after operation.
- Burn injuries wound infection

- Refusal to give consent for participating in the study

4.6 Sample Size

The sample size was calculated based on single sample size estimation as shown below [40]. The value of p was taken as 88.1% (0.881) from the study conducted by Aschalew *et al* on Isolation of bacterial pathogens from patients with postoperative surgical site infections and possible sources of infections at the University of Gondar Hospital, Northwest Ethiopia [37].

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

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion (P = 0.5), and d = precision (d = 0.05), Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

$\frac{(1.96)^2 \times 0.881(1-0.881)}{(0.05)\ 2} = \frac{161.}{}$ The 10 % non response rate is 16.1.

Therefore the minimum sample size was: 177

4.7 Sampling Method and procedure

A total of 197 study participants was recruited using convenient sampling technique.

4.8 Study Variables

4.8.1 Dependent Variables

- Prevalence of bacterial isolates
- Antibiotic Susceptibility pattern of bacterial isolates

4.8.2 Independent Variables

- Age
- Sex

- Patient status (Outpatient or inpatient)
- Wards
- Wound type

4.9 Data Collection

4.9.1 Socio-demographic data

After obtaining informed consent socio-demographic data (age, sex, ward and patient type) of patients were collected.

4.9.2 Wound (pus) sample Collection

Those eligible surgical patients were subjected to daily surveillance for the development of infection. From these surgical patients who developed postoperative wound infections wound swabs were taken for microbiological follow up. The wound swabs were collected by the principal investigator and nurses with guiding by resident doctors using data collection form and sterile cotton swab on sterile transport media. Patients were identified from the operating room log and daily operation schedule. Patient specific demographic characters and information on potential predicators of wound infection including the health condition and wound class were recorded on the day of the operation from the patient card and the responsible surgeon. Patients were followed for the development of wound infection by the principal investigator. In addition each day the responsible ward nurse/resident was approached those to find out patient with wound infection [41].

4.10 Transport of specimens

All post operative wound swabs were dipped in Stuarts transport media immediately after collection and taken to the bacteriology laboratory for culturing.

4.11 Culture and Identification

The wound swab specimens were inoculated on blood agar, MacConkey agar and Mannitol salt agar and were incubated at 37^{o}C for 24 hours. Identification of bacterial isolates was performed

using colony morphology, Gram-stain and conventional biochemical tests. For all isolates antimicrobial susceptibility testing was carried out.

4.12 Drug Susceptibility Patterns

The disk diffusion method was performed and after 16-18hours of incubation at 37^{o}C zone of inhibition was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) [42]. Using a sterile wire loop, 3-5 pure colonies were picked from blood for Gram positive and MacConkey agar for Gram negative and emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland was swabbed onto Muller-Hinton agar (dispensed on 100mm plate). Drug susceptibility testing of all bacteria isolates was performed using disk diffusion method incubating at 37^{o}C for 24 hours. In common all bacteria isolates were tested against Amoxicillin-Clavulanic acid (30µg, BD), Chloramphenicol (30µg, BD), Gentamycin (10µg, BD), TMP-SXT (1.25µg+23.75µg, BD), Tetracycline (30µg, BD) and Ciprofloxacin (5µg, BD). Additionally all Gram positive isolates were tested for Penicillin, oxacillin (5µg, BD), Clindamycin(2µg, oxoid) and Erythromycin(15 µg oxoid). In addition to the common drugs gram negative isolates were tested against Amoxicillin (30µg, BD), Cefotaxime (30µg, BD), Ceftazidm, Cefriaxone and Amikacin. (10µg, Oxoid). The zone of inhibition was measured to the nearest millimeter and isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI. High, intermediate and low level of resistance was interpreted when the percentage of resistance was >80%, 60-80% and<60% respectively [43].

4.12.1 Quality Control

Standard Operating Procedures (SOP) were strictly followed verifying that media meet expiration date and quality control parameters per CLSI [42]. Visual inspections of cracks in media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination was done. QC was performed to check the quality of medium. Each new lots were quality controlled before use by testing the *E. coli ATCC 25922* and/or *Staphylococcus aurous ATCC 25923* standard control strains.

4.13 Statistical analysis and interpretation

The data was entered and analyzed using SPSS version 20 double checked before analysis. The descriptive statistics (mean, percentages or frequency) was calculated. The bi-variant logistic regression analysis was used to see the relation between dependent variable and independent variables. Variables that showed a significant association were selected for further analysis using multiple logistic regression models with a p-value < 0.05 as statistically significant. Finally, the results were presented in words, charts, graphs and tables.

4.14 Data quality Assurance

Socio-demographic characteristics of the patient were collected appropriately after getting informed consent. Samples were collected in accordance with SOPs and taken soon for analysis. Culture results were recorded carefully before entry to statistical tool. Before analysis the data was double checked.

4.15 Operational definitions

i. **Postoperative wound infection:** is a type of healthcare associated infection in which wound infection occurs at the site of surgery.
ii. **Multidrug Resistance(MDR)** is a bacterium that is simultaneously resistant for two or more antimicrobials belonging to different chemical classes

4.16 Ethical clearance

The study was approved by "Department Research and Ethical Review Committee (DRERC)" of the Department of Medical Laboratory Science (MLS/483/15), School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. Permission letter was also obtained from the study site. The purpose and procedures of the study was explained to the study participants, participants' parents or guardians within the study period. Those participants who gave informed consent and children who gave assent and whose parents or guardians gave informed consent were selected and enrolled as the participants of the study. A patient result was communicated to the attending physicians.

5 Results

5.1 Socio-demographic characteristics

One hundred ninety seven (n=197) study participants were investigated during the study period. Of these patients, 59.9% (n=118/197) were males and 40.1% (n=79/197) were females with males to females ratio 1.49:1. The majority of patients 53(26.9%) and 51(25.9%)were between 1-10 and 21-30 years of age as shown in figure 5.1 and the mean (std. deviation) ages of patients were 24.8(19.2) with age range of 10 days-85 years. 14.7% (n=29/197) were out patients while the remaining 85.3% (n=178/195) were inpatients. Socio-demographic characteristics of patients have shown in table 5.1.

Table 5.1 Socio-demographic characteristics of patients who develop postoperative wound infections at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015-

Variables		Culture results (n=197)		
		Positive n (%)	**Negative n (%)**	**Total n (%)**
Gender	Male	93(78.8)	25(38.8)	118(100)
	Female	56(70.9)	23(29.1)	79(100)
	Total	**149(75.6)**	**48(24.4)**	**197(100)**
Age in Year	<10	42(79.3)	11(20.7)	53(100)
	11-20	29(82.9)	6(17.1)	35(100)
	21-30	36(70.6)	15(19.4)	51(100)
	31-40	14(77.8)	4(22.2)	18(100)
	41-50	14(73.7)	5(26.3)	19(100)
	>50	14(66.7)	7(23.3)	21(100)
	Total	**149(75.6)**	**48(24.4)**	**197(100)**
Patient Type	Outpatient	24(82.8)	5(17.3)	29(100)
	Inpatient	125(74.4)	43(25.6)	168(100)
	Total	**149(75.6)**	**48(24.4)**	**197(100)**
Wound status	Dirty	12(100)	0(0)	12(100)
	Contaminated	58(98.3)	1(1.7)	59(100)
	Clean contaminated	63(78.8)	17(21.2)	80(100)
	Clean	16(34.8)	30(65.2)	46(100)
	Total	**149(75.6)**	**48(24.4)**	**197(100)**
Wards	Orthopedics	42(82.4)	9(17.6)	51(00)
	Intensive Care Unit	9(81.8)	2(18.2)	11(100)
	General surgery	37(75.5)	12(24.5)	49(100)
	Gynecology & obstetrics	13(68.4)	6(31.6)	19(100)
	Internal Medicine	4(57.1)	3(42.9)	7(100)
	Pediatrics	20(64.5)	11(35.5)	31(100)
	Total	**149(75.6)**	**48(24.4)**	**197(100)**

5.2 Prevalence of bacteria Isolates from postoperative wounds

The overall prevalence of bacteria isolates from postoperative wound infections was 75.6% (n=149/197). Among this 62.4% (n=93/149) of the culture positives were from males and 37.6% (n=56/149) were from females. Gram positive and Gram negative isolates constitutes 45.2% (n=76/168) and 54.8% (n=92/168) respectively with Gram positives to Gram negatives ratio of 0.83:1. The predominant bacteria isolated from cultures were *Staphylococci aureus33.3%* (n=56/168), *Escherichia coli 14.3% (n=24/168, CONS* 11.3% (n=19/168), *Acinetobacter species 10.1% (n=17/168), Klebiesella pneumoniae* 8.9% (n=15/168, *Pseudomonas species 5.3% (n=9/168) and Pseudomonas aeroginosa 4.8% (n=8/168*) (figure 5.1). Among culture positive results 11.4% (n=17/149) double infections were seen and the highest proportion were from orthopedics 52.9% (n=9/17)(table 5.2).

The spectrum of post operative wound infection varies with the age of patients (table 5.1). 28.2% (n=42/149) of post operative wound infections were found in less than 10 years who share the highest proportion post operative wound infections. However there was no significant association between age of patient and culture results (OR=1.13, 95%CI=0.931-1.377, P = 0.212) (table 5.4).

In this study 83.9% (n=125/149) of bacteria were isolated from hospitalized patients while the remaining 16.1% (n=24/149) were from those who attended outpatient department; however there was no significant association between being out patient or inpatient on post operative wound infection culture results (OR=1.653, 95%CI=0.593-4.597, P = 0.337). And from hospitalized patients the highest proportion of bacteria 28.2% (n=42/149) were isolated from orthopedics which shares the highest proportion 25.9% (n=51/197) among the different wards. However there was no significant association between different wards and culture results (OR=1.03, 95%CI=0.309-3.424, P = 0.936). While General Surgery 24.8% (n=37/149) and OPD 16.1 % (n=24/149) took the second and third rank pediatrics 13.4% (n=20/149), gynecology and obstetrics 8.7% (n=13/149) and internal medicine 2.7% (n=4/149) follows.

In our study monomicrobial isolates were recovered from 88.6% (n=132/149) patients whereas 11.4% (n=17/149) had polymicrobial infections. From polymicrobial infections *S. aureus* and *Pseudomonas species* were the most isolated 23.5% (n=4/17) followed by *S.*

aureus and *E. coli* 17.6% (n=3/17) table 5.3. Highest polymicrobial infections 52.9% (n=9/17) were showed from orthopedics ward (table 5.2).

Table 5.2 Rate of single and multiple infections from postoperative wounds among different wards at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

	Culture results			
Wards	**Single infection**	**Double infection**	**No-Growth**	**Total**
Orthopedics	33(64.7%)	9(17.6%)	9(17.6%)	51(100%)
ICU	6(54.5%)	3(27.3%)	2(18.2%)	11(100%)
General surgery	35(71.4%)	2(4.1%)	12(24.5%)	49(100%)
Gynecology & Obstetrics	13(68.4%)	0(0.0%)	6(31.6%)	19(100%)
Internal medicine	3(42.9%)	1(14.3%)	3(42.9%)	7(100%)
Pediatrics	19(61.3%)	1(3.2%)	11(35.5%)	31(100%)
Out patient	23(79.3%)	1(3.4%)	5(17.2%)	29(100.0%)
Total	132(67.0%)	17(8.6%)	48(24.4%)	197(100.0%)

Table 5.3 Frequency of double infections from patients who develop postoperative wound infections at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

Double infections		
	Frequency	%
K. Pneumoniae and E.coli	1	5.9
Acinetobacter spp and E.coli	1	5.9
Acinetobacter spp and Citrobacter diversie	1	5.9
S.aureus and Pseudomonas spp	4	23.5
S.aureus and Acinetobacter spp	1	5.9
S. aureus and E. coli	3	17.6
S. aureus and K. pneumoniae	2	11.8
P. mirabiles and K. rhinoscleris	1	5.9
E. coli and Citrobacter spp	1	5.9
E. coli and Providencia stuartii	1	5.9
Citrobacter spp and K. rihinoscleris	1	5.9
Total	**17**	**100.0**

Among the different wound status of postoperative wound infections clean contaminated wound took the highest proportion 40% (n= 80/197) from all wound status. Among culture positives it yields the highest culture results 42.3% (n=63/149) followed by contaminated wound 25.5% (n=38/149) and clean wound 10.7% (n=16/149). However there was no significant association between wound status of the patient and culture results (OR=0.109, 95%CI=0.43-0.274, P = 0.998). Dirty wounds yield 100% (n=12/12) positive culture results though it took the least proportion 6.1% (n=12/197) from all wound status.

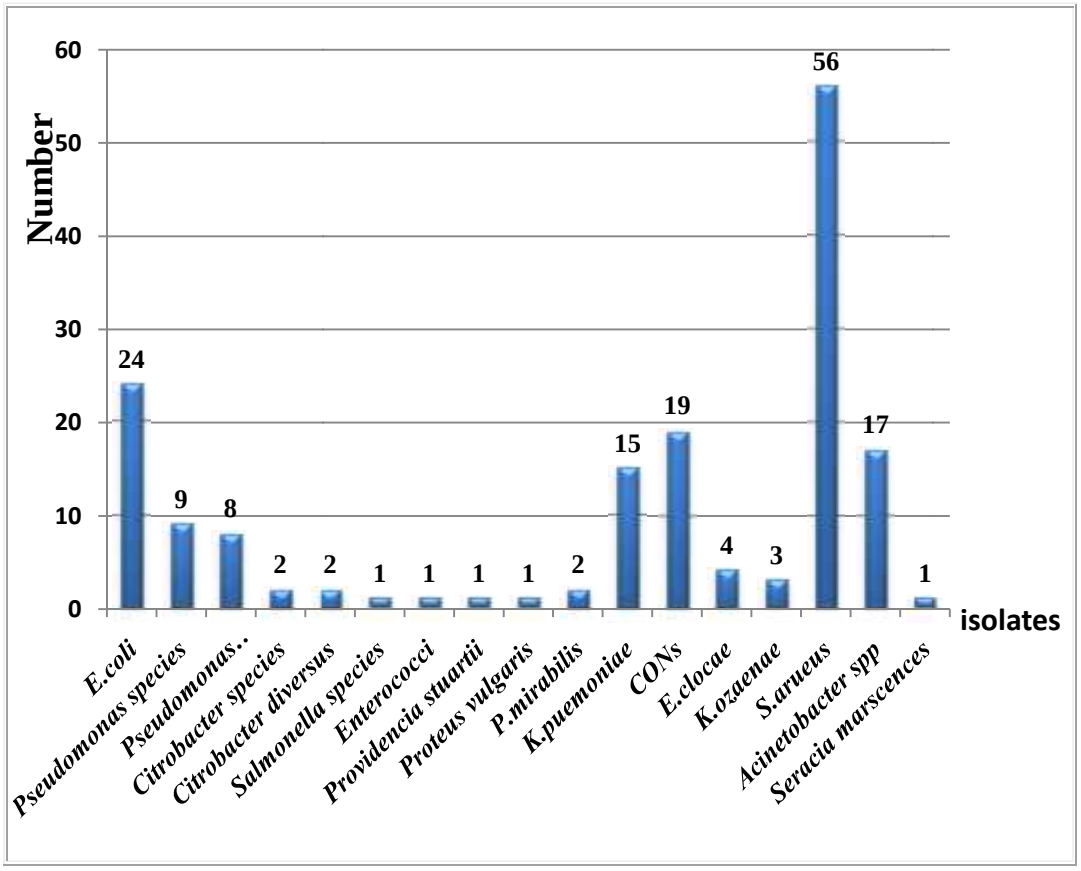

Figure 5.2 Frequency and type of bacteria isolated from postoperative wounds at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

Table 5.4 Association of variables with postoperative wound culture results at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

Variables		Culture results (n=197)		P-value	OR	95%CL	P-value	AOR	95%CI
		Positive n(%)	Negative n(%)						
Gender	Male	93(78.8)	25(38.8)	0.205	0.655	[0.340-1.262]			
	Female	56(70.9)	23(29.1)	1					
	Total	**149(75.6)**	**48(24.4)**						
Age in Year	<10	42(79.3)	11(20.7)	0.260	0.524	[0.170-1.612]			
	11-20	29(82.9)	6(17.1)	0.1711	0.414	[0.117-1.464]			
	21-30	36(70.6)	15(19.4)	0.743	0.833	[0.280-2.476]			
	31-40	14(77.8)	4(22.2)	0.445	0.571	[0.136-2.399]			
	41-50	14(73.7)	5(26.3)	0.629	0.714	[0.182-2.800]			
	<50	14(66.7)	7(23.3)	1					
	Total	**149(75.6)**	**48(24.4)**						
Patient Type	Outpatient	24(82.8)	5(17.3)	1					
	Inpatient	125(74.4)	43(25.6)	0.337	1.651	[0.593-4.597]			
	Total	**149(75.6)**	**48(24.4)**						
Wound status	Dirty	12(100)	0(0)	0.998	0.000	0.000			
	Contaminated	58(98.3)	1(1.7)	0.000	0.009	[0.001-0.073]	0.000	0.008	[0.001-0.063]
	Clean contaminated	63(78.8)	17(21.2)	0.000	0.144	[0.064-0.323]	0.000	0.133	[0.056-0.318]
	Clean	16(34.8)	30(65.2)	1			1		
	Total	**149(75.6)**	**48(24.4)**						

OR--Odds ratio, CI--Confidence Interval, AOR--Adjusted odds ratio, NA---Not applicable

5.3 Antibiotic Resistance patterns

Among the total isolates (n=168) multidrug resistance (MDR ≥2 different classes of drugs) were recorded in 65.5% (n=110/168) of all bacterial isolates. Gram positive and Gram negative isolates showed 55.3% (n=42/76) and 73.9% (n=68/92) multiple drug resistance respectively. Antimicrobial resistance level for the Gram positive isolates causing postoperative wound infections ranges from 0-100% with multiple drug resistance of 55.3% (n=42/76). Among the Gram positive bacteria the predominant isolate *Staphylococci aurous* 73.7% (n=56/76) showed 44.6% (n=25/56) of MDR. It demonstrated high level of resistance to Penicillin (80.4%) and lower resistance level for Sulphamethoxazole-Trimethoprim (33.9%), Tetracycline (28.6%) and Erythromycin (16.1%). It showed better susceptibility for Clindamycin (1.8%), chloramphenicol (7.1%) and oxacillin (10.7%) compared to other tested drugs (table 5.5). The second dominant Gram positive bacteria was *coagulase negative staphylococci* 25% (n=19/76) which showed 84.2% (n=16/19) of MDR level. It showed high level of resistance to Penicillin (94.7%) and lower resistance level for Sulphamethoxazole-Trimethoprim (33.9%), Tetracycline (28.6%) and Erythromycin (16.1%). It showed better susceptibility for Ciprofloxacin (26.3%) and Clindamycin (31.6%).

In the same manner the resistance patterns of Gram-negative organisms causing postoperative wound infections were ranging from 0 - 100% and they showed 73.9% (n=68/92) multiple drug resistance (table 5.6). Almost all isolates showed greater than 90% level of resistance for amoxicillin and variable level of resistance for other drugs and almost all isolates were sensitive for Amikacin (>95%). Among the Gram negative bacteria the predominant isolates *E. coli* 24.1% (n=24/92) showed 21(87.5%) of multidrug resistance level which demonstrates high level of resistance to amoxicillin (90%) and intermediate resistance level for Cefotaxime (62.5%), Sulphamethoxazole-Trimethoprim (62.5%) and Tetracycline (62.5%). It showed lower resistance level for chloramphenicol (16.7%) and Gentamycin (29.2%)(Table 5.5).

Among the antibiotics tested amoxicillin (93.5%), Ceftriaxone (85.3%), Penicillin (84.5%) and Cefotaxime (82.7%) showed high level of resistance. Clindamycin (7.9%) and Amikacin (1.1%) showed high sensitivity for Gram Positive and Gram negative respectively.

Table 5.5 Antimicrobial resistance levels of bacterial isolates from postoperative wounds at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

Organism isolated	**Antimicrobial resistance level in no.(%)of bacterial isolates from postoperative wounds**															
	AML	**AMC**	**SXT**	**PEN**	**CLD**	**VA**	**OX**	**ERT**	**GE**	**CTX**	**CAZ**	**C**	**TE**	**Amk**	**CIP**	**CRO**
S. aureus (n=56)	NT	9(16.1)	19(33.9)	45(80.4)	1(1.8)	NA	6(10.7)	9(16.1)	7(12.5)	NA	NA	4(7.1)	16(28.6)	NA	7(12..5)	NT
K. ozenae(n=3)	3(100)	3(100)	3(100)	NA	NA	NA	NA	NA	3(100)	3(100)	3(100)	2(66.7)	3(100)	0(0)	2(66.7)	3(100)
P. mirabilis (n=2)	2(100)	2(100)	2(100)	NA	NA	NA	NA	NA	2(100)	1(50)	0(0)	2(100)	2(100)	0(0)	0(0)	1(50)
K. rihinoscleris (n=2)	2(100)	2(100)	2(100)	NA	NA	NA	NA	NA	0(0)	2(100)	2(100)	1(50)	2(100)	0(0)	2(100)	2(100)
S. marscensence (n=1)	1(100)	1(100)	1(100)	NA	NA	NA	NA	NA	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)
Citrobacter spp (n=2)	1(50)	1(50)	1(50)	NA	NA	NA	NA	NA	1(50)	1(50)	1(50)	0(100)	1(50)	0(0)	1(50)	1(50)
C. diversus (n=2)	2(100)	2(100)	1(50)	NA	NA	NA	NA	NA	2(100)	2(100)	2(100)	1(50)	1(50)	0(0)	2(100)	2(100)
Enterococci (n=1)	NA	NA	NA	1(100)	NA	0(0)	NA	NA	NA	NA	NA	1(00)	1(100)	NA	NA	NA
P. stuartii (n=1)	1(100)	1(100)	1(100)	NA	NA	NA	NA	NA	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)
Salmonella spp (n=1)	1(100)	1(100)	1(100)	NA	NA	NA	NA	NA	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)
K. pneumoniae (n=15)	14(93.3)	12(80)	13(86.7)	NA	NA	NA	NA	NA	9(100)	13(86.7)	9(60)	7(46.7)	9(60)	0(0)	4(26.7)	13(86.70)
P. aerogens (n=8)	8(100)	7(87.5)	7(87.5)	NA	NA	NA	NA	NA	2(25.0)	NA	1(12.5	3(37.5)	8(100)	1(12.5)	1(12.7)	NA
Pseudomonas spp (n=9)	9(100)	8(88.9)	8(88.9)	NA	NA	NA	NA	NA	7(77.8)	NA	6(66.7)	7(77.8)	9(100)	0(0)	3(33.3)	NA
Acinetobacter spp(n=17)	17(100)	15(88.2)	15(88.2)	NA	NA	NA	NA	NA	12(70.6)	17(100)	16(94.1)	13(76.5)	14(82.4)	0(0)	12(70.6)	17(100)
E. coli (n=24)	20(83.3)	14(58.3)	15(62.5)	NA	NA	NA	NA	NA	7(29.2)	15(62.5)	14(58.3)	4(16.7)	15(62.5)	0(0)	9(37.5)	17(70.8)
CONS (n=19)	NT	15(78.9)	14(73.7)	18(94.7)	6(31.6)	NA	10(52.6)	11(57.9)	12(63.2	NA	NA	9(47.4)	12(63.2)	NA	5(26.3)	NA
P. vulgaris (n=1)	1(100)	1(100)	1(0)	NA	NA	NA	NA	NA	1(100)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)
E. cloacae (n=4)	4(100)	2(50)	1(25)	NA	NA	NA	NA	NA	1(25)	4(100)	4(100)	1(25)	1(25)	0(0)	1(25)	4(100)
Total(n=168)	**86(93.5)**	**96(57.5)**	**105(62.9)**	**64(84.2)**	**7(9.3)**	**0(0)**	**16(21.3)**	**20(26.7)**	**68(40.7)**	**62(82.7)**	**49(53.3)**	**57(33.9)**	**97(57.7)**	**1(1.1)**	**50(29.8)**	**64(85.3)**

NA--Not applicable: NT--Not Tested, AML--Amoxycillin, AmC--Amoxycillin-Clavulanic acid, Amk--Amikcin, SXT—Sulphamethoxazol-trimethoperem, C--Chloramphenicol, CTX--Cefotaxime, CAZ-Ceftazidime, CRO-cetfriaxone, GM--Gentamycin, TE--Tetracycline, CIP--ciprofloxacin, OX--Oxacillin, ERY--Erythromycin, CLD--Clindamycin, VA-Vancomycin, PEN--Penicillin

Table 5.6 Multidrug resistance pattern of bacteria isolated from postoperative wounds at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

Organism isolated	**Antimicrobial resistance level in no.(%)of bacterial isolates**									
Gram Positive	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**	**Total**
S. aureus (n=56)	8(14.3)	6(10.7)	3(5.4)	4(7.1)	1(1.8)	1(1.8)	0(0)	2(3.6)	0(0)	25(44.6)
CONS (n=19)	1(5.3)	2(10.5)	2(10.5)	0(0)	1(5.3)	1(5.3)	4(21.1)	4(21.1)	1(5.3)	16(84.2)
Enterococci (n=1)	(0)	1(100)	0(0)	-	-	-	-	-	-	1
Total *(n=76)*	**9(11.8)**	**9(11.8)**	**5(6.6)**	**4(5.3)**	**2(2.6)**	**2(2.6)**	**4(5.3)**	**6(7.9)**	**1(1.3)**	**42(55.3)**
Gram Negative										
K. ozaenae (n=3)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	1(33.3)	1(33.3)	3(100)
P. mirabilis (n=2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)	0(0)	0(0)	2(100)
K. rihinoscleris (n=2)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)	0(0)	0(0)	2(100)
S. marscensence (n=1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	(0)	0(0)	1(100)
Citrobacter spp (n=2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)
Citrobacter diversus (n=2)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)		2(100)
Providencia stuartii (n=1)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)
Salmonella spp (n=1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)
K. pneumoniae (n=15)	0(0)	0(0)	2(13.3)	4(26.7)	0(0)	2(13.3)	4(26.7)	2(13.3)	0(0)	14(93.3)
P. aerogens (n=8)	1(12.5)	1(12.5)	0(0)	0(0)	-	-	-	-		2(25)
Pseudomonas spp (n=9)	3(33.3)	3(33.3)	0(0)	0(0)	0(0)	0(0)	-	-		6(66.7)
Acinetobacter spp (n=17)	1(5.9)	1(5.9)	1(5.9)	4(23.5)	0(0)	0(0)	-	-		7(41.2)
E. coli (n=24)	1(4.2)	2(8.3)	2(8.3)	3(12.5)	2(8.3)	2(8.3)	6(25)	2(8.3)	1(4.2)	21(87.5)
P. vulgaris (n=1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)
E. cloacae (n=4)	1(25)	1(25)	1(25)	0(0)	0(0)	0(0)	0(0)	1(25)	0(0)	4(100)
Total (n=92)	**7(7.6)**	**8(8.7)**	**6(6.5)**	**12(13.0)**	**3(3.3)**	**6(6.5)**	**17(18.5)**	**7(7.6)**	**2(2.2)**	**68(73.9)**

6 Discussion

6.1 Prevalence of bacteria isolates among postoperative wound infections

The overall prevalence of bacteria isolates from postoperative wound infections was 75.6% (n=149/197) showed exact similarity with a study done in Ethiopia [44]. This finding was almost similar result with what had been reported previously in Ethiopia (71.1%) [21]. To the contrary lower rates of isolation (11.4%, 10.9%) were reported from localities of Ethiopia [25, 35]. However, this finding was relatively lower than studies done in other parts of Ethiopia (92%, 87.3%) [34, 36] and much lower than a study done in India (96%) [45].The possible explanation for the difference could be varying distribution of bacterial etiology with geography, time and infection prevention practice in diverse settings. Difference in identification methods are also known to influence the relative prevalence of bacteria which makes comparison of results difficult [21]. The absence of bacterial growth in samples collected from apparently infected surgical wounds could be due to the effect of antimicrobials which are used routinely in surgery, it can also be due to antiseptics used for cleaning the wounds or it may even be due to the body's defense mechanism overcoming the infection. It is also possible that some organisms were unable to grow under the aerobic condition the samples were cultured like anaerobic bacteria [34, 35].

The incidence of wound infection was more common in males 62.4% (n=93/149) than in females 37.6% (n=56/149). However there was no significant association between sex of patient and culture results (OR=0.65, 95%CI=0.340-1.262, P = 0.205).This is in agreement with studies done in different parts of Ethiopia [21, 36, 46] and other countries [47]. This might be explained by the fact that traditionally, in this country mainly males are involved in occupations such as farming, construction works, transportation and industry works where the likely exposure to trauma is common [36]. Another reason may be that males are highly exposed to external environment than female [46]. The spectrum of post operative wound infection varies with the age of patients (table 5.1). 28.2% (n=42/149) of post operative wound infections were found in less than 10 years who share the highest proportion post operative wound infections in contrary to other study[46]. However there was no significant association between age of patient and culture results (OR=1.13, 95%CI=0.931-1.377, P = 0.212). It showed similarity with other studies [24, 47] and disagreement with a study done in Ethiopia which reported that age of the

patient showed significant association with culture results[26]. In this study 83.9% (n=125/149) of bacteria were isolated from hospitalized patients while the remaining 16.1% (n=24/149) were from those who attended outpatient department; however there was no significant association between being out patient or inpatient on post operative wound infection culture results (OR=1.653, 95%CI=0.593-4.597, P = 0.337). This is a similar finding with a study done in Ethiopia [21].

Among the different wards the highest proportion of bacteria 28.2% (n=42/149) were isolated from orthopedics which shares the highest proportion 25.9% (n=51/197) among the different wards which has similarity with other study [44]. However there was no significant association between different wards and culture results (OR=1.03, 95%CI=0.309-3.424, P = 0.936). While General Surgery 24.8% (n=37/149) and OPD 16.1 % (n=24/149) took the second and third rank pediatrics 13.4% (n=20/149), gynecology and obstetrics 8.7% (n=13/149) and internal medicine 2.7% (n=4/149) follows. It also showed similarity with other studies done in different parts of the world [46]. This could be due to in Orthopedic wards, since patients require longer hospitalization time to recover from their bone cases, they become prone for post operative wound infections [46].

In our study among the different class of wound of postoperative wound infections clean contaminated took the highest proportion 40% (n= 80/197) from all wound status which also yield highest culture results 42.3% (n=63/149). Multivariate regression analysis showed that there was significant association between wound status of the patient and culture results (OR=0.144, 95%CI=0.0640-0.323, P = 0.00). It showed agreement with other study done in Ethiopia [21]. This high rate of infection would be probably because of profound influence of endogenous contamination during the time of operation [21]. Contaminated wound 58.9% (n=38/149) and clean wound 10.7% (n=16/149) took the second and third culture positive ranks following to clean contaminated wound. In case of dirty wounds it yields 100% (n=12/12) positive culture results though it took the least culture positive proportion 8.1% (n=12/149) from all wound status. However there was no significant association between wound status of patient culture results (OR=0.000, 95%CI=0.0640-0.323, P = 0.998). It showed an agreement with a study done in Ethiopia [44].

Multiple infections among culture positive post surgical wound infected patients was seen in 11.4% (n=17/149) of patients where *S. aureus* and *Pseudomonas species* showed common association where as the rest 88.6% (n=132/149) have shown single bacterial infection. The rate of poly-microbial pathogens in the current study (11.4%) was comparable with a study done in South West Ethiopia [36] however it was a lower finding compared to another study conducted in Southern Ethiopia (20.1%) [21].The leading double infections caused by *S. aureus* and *Pseudomonas species* similar with a study done in other parts of Ethiopia [26].

In our study Gram negatives 54.8% (n=92/168) were isolated more commonly than Gram positive isolates 45.2% (n=76/168) which showed similarity with other studies [36, 48, 49, 50]. The most predominant bacteria isolated in this study were *Staphylococci aurous 33.3%* (n=56/168). It showed similarity with other studies done throughout the world [21, 36, 49, 51].

The predominance (33.3%) of *S. aureus* infection seen in this study is most likely associated with endogenous source as the organism is a member of the skin and anterior nares of the patients which can enter to deep site during surgery of the natural barrier of the skin could be the possible justification for its high prevalence [44]. Infection with this organism may also be associated with contamination from the environment, surgical instruments or contaminated hands of the health professionals [35]. However it showed disagreement with a study done in Nepal where Pseudomonas species were the most isolated bacteria from postoperative wound infections [46]. The possible reason for variation in the studies could be attributed to differences in the populations investigated; diversity of surgical procedures performed on the study participants, as well as timing of specimen collections [46]. The second predominant bacteria isolated in this study was *Escherichia coli 14.3% (n=24/168).* It showed an agreement with a study done in Nepal where the second dominant bacteria isolated was *E. coli* which showed a comparable finding (17.5%) [46].This could be because of the profound influence of endogenous contamination from the bowel and hollow muscular organs of patients [35]. Other bacteria isolates in this study were *CONS* 11.3% (n=19/168), *Acinetobacter species 10.1% (n=17/168), Klebsiella pneumoniae* 8.9% (n=15/168, *Pseudomonas species 5.3% (n=9/168) and Pseudomonas aeroginosa 4.8% (n=8/168*).

6.2 Antimicrobial resistance patterns of bacterial isolates

The overall multidrug resistance level of all bacteria isolates was 65.5% (n=110/168). This finding was a similar result 65.2% with the study done in other parts of Ethiopia; Bahr Dar [26].Our finding was relatively lower than what has been recorded in South West Ethiopia which was 85.2% [36]. In our study Gram positive and Gram negative bacteria showed 55.3% (n=42/76) and 73.9% (n=68/92) multiple drug resistance respectively. The high frequency of multiple antibiotics resistance might be a reflection of inappropriate use of antimicrobials, lack of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics, unskilled practitioners, expired antibiotics, self medication, and counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates [54].

Majority of the Staphylococci were multiple antibiotics resistant and these multi-drug resistance patterns had been documented already [55]. The predominant isolates *Staphylococci aurous* showed an overall MDR of 44.6%(n=25/56) which showed disagreement with a study done in Hawassa and Bahr Dar, Ethiopia[34, 35] where *Staphylococci aurous* showed 100% MDR level. The difference could be due to the difference in prescribing this antibiotic for the treatment of the bacteria from hospital to hospital [44]. It demonstrated high level of resistance to Penicillin (80.4%) which was consistent with study done in other part of Ethiopia [36] and relatively lower resistance level for Sulphamethoxazole-Trimethoprim (33.9%), Tetracycline (28.6%) and Erythromycin (16.1%). It showed better susceptibility for Clindamycin (1.8%), Chloramphenicol (7.1%) and oxacillin (10.7%) compared to other tested drugs.

Among the Gram negative bacteria the predominant isolates *E. coli* 24.1% (n=24/92) showed 21(87.5%) of multidrug resistance level which demonstrates high level of resistance to amoxicillin (90%) and intermediate resistance level for Cefotaxime (62.5%), Sulphamethoxazole-Trimethoprim (62.5%) and Tetracycline (62.5%). It showed lower resistance level for chloramphenicol (16.7%) and Gentamycin (29.2%). This finding showed disagreement with a study done in Ethiopia where *E. coli* isolates showed high resistant level to ampicillin (96.6%), tetracycline (79%), chloramphenicol (65.5%), ceftriaxone (62%), sulphamethoxazole trimethoprim (55%) and gentamicin (51.7%). The difference could be due to the difference in prescribing this antibiotic for the treatment of the bacteria from hospital to hospital [44]. High resistance rates were recorded to amoxicillin (93.5%), Ceftriaxone(85.3%), Penicillin (84.2%) and Cefotaxime(82.7%) .

Limitations of the study

- The study did not isolate strict anaerobes bacteria and fungi which could have increased the number of bacterial isolates reported as negative cultures.

7 Conclusion

The results of this study showed the prevalence and antimicrobial resistance patterns of postoperative wound infections over a period of eight month. The overall prevalence of bacteria isolates from postoperative wound infections was 75.6% (n=149/197) and the predominant isolated bacteria were *Staphylococci aureus33.3%* (n=56/168) and *Escherichia coli 14.3% (n=24/168).* Although complete eradication of postoperative wound infections is not possible, precautions should be taken to minimize by strictly adhering aseptic surgical procedures and proper management of wounds.

The choice of drugs in the treatment of bacterial isolates from postoperative wound infections is quite narrow today due to the wide scale resistance of common pathogens to drugs which have been used previously. Multidrug resistances were recorded in 65.5% (n=110/168) of all bacterial isolates. Gram positive and Gram negative isolates showed 55.3% (n=42/76) and 73.9% (n=68/92) multiple drug resistance respectively. The predominant isolates *S. aureus* and *E. coli* showed 44.6% (n=25/56) and 21(87.5%) of MDR respectively. Among the antibiotics tested amoxicillin (93.5%), Ceftriaxone (85.3%), Penicillin (84.5%) and Cefotaxime (82.7%) showed high level of resistance. Moreover, bacterial strains resistant to most classes of antibiotics arise which is our current challenge.

8 Recommendations

- Routine microbial analysis of postoperative wound infections and use of their antibiotics is recommended so as to guide clinician for the treatment of postoperative wound infections.
- A regular monitoring of antimicrobial resistance patterns in post-operative wound infections are essential and mandatory to prevent further emergence and spread of antimicrobial resistance among bacterial pathogens especially in the case of multidrug resistance bacteria.
- Effective antibiotic policy like antibiotic restriction, combination therapy and antibiotic cycling, and infection control programs combined with good medical practices can help in confronting the menace of antibiotic resistance to prevent the spread of multidrug resistant strains.

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Annexes I

Annex I: Laboratory procedures for sample collection, biochemical reactions, drug susceptibility testing (CLSI guidelines).

1. Sample collection

1. Using a sterile technique, aspirate or collect from a drainage tube up to 5 ml of pus. Transfer to a leak-proof sterile container. But when pus is not being discharged , use a sterile cotton-wool swab to collect a sample from the infected site .
2. Label the specimen and as soon as possible deliver it with a completed form to the laboratory

2. Culture and identification

i. Bacteria growth from the blood and MacConkey agar

Positive/present □ Negative/absent □

ii. Identification steps for suspected colonies

a) Gram stain result _____
b) Oxidase test positive □ /negative □
c) Colony morphology
d) Lactose fermentation from MacConkey agar
   - Lactose Fermenter
   - Late Lactose fermenter
   - Non lactose fermenter

iii. Biochemical reactions

Identification of bacterial isolates involves the use of biochemical screening Medias. Indole, Urease, Mannitol, Triple sµgar iron (TSI), Citrate, Motility, Lysine Decarboxylase, Mannolet and Oxidase tests.

Biochemical reactions		Indol	Urea	Man	TSI	Cit	Mot	LDC	OX
Result	Positive								
	Negative								
Gram negative rods									

Key: LDC = Lysine decarboxylase, Man = Mannitol (mannite), Triple sµgar iron (TSI), Ox = Oxidase test, Cit = Citrate test, Mot = Motility, Ind = Indole test, Urea = Urease, H2S = Hydrogen sulphide (blackening), R = Red-pink (alkaline reaction), Y = Yellow (acid reaction), d = different strains give different results.

A. **Indole test**: Few colonies of the culture will be inoculated into peptone water and incubated at 37oC for 24 hours. Few drops of indicator (Kovac's reagent) will be added and gently shake to mix well. Colour change will be then observed. If the layer of indicator reagent turns to red within 1 minute, it is Indole positive (positive result). If the layer of indicator reagent remains yellow within 1 minute, it is indole negative (negative result).
B. **Urease test (Christensen's (modified) urea broth)**: Urea agars will be inoculated heavily over the entire surfaces of the slants in bijou bottles. The cap will be loosened and then incubated at 37oC for 3-12 hours. A urease-positive culture produces an alkaline reaction in the medium, evidenced by pinkish red color of the Medium. Urease-negative organisms do not change the color of the medium, which is pale yellow-pink.
C. **Triple Sugar Iron (TSI) Agar Slant:** Using a sterile inoculating needle, stab the butt of the LIA slant twice then streak back and forth along the surface of the agar with the organism. Incubate at 37◦C for 18 to 24 h. If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented. If alkaline slant–acid butt (red–yellow): glucose fermented only. If alkaline slant–alkaline butt (red–red): glucose not fermented. The presence of black precipitate (butt) indicates hydrogen sulfide production, and presence of splits or cracks with air bubbles indicates gas production.
D. **Citrate utilization test using Simmon's citrate agar**: Simmon's citrate slopes will be prepared in bijou bottles as recommended by the manufacturer (stored at 2-8oC). And the slopes will be then stabbed and incubated at 37oC aerobically for 48 hours. Blue colour

indicates a positive reaction and if Simmon's citrate agar slopes remained as green in colour indicate negative reaction.

E. **Motility Test (using motility agars)**: Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35-37oC for 24 hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation.

F. Lysine decarboxylase: Decarboxylation of lysine can be detected by culturing bacteria in a medium containing the desired amino acid, glucose, and a pH indicator bromcresol purple. The acids produced by the bacteria from the fermentation of glucose will initially lower the pH of the medium and cause the pH indicator to change from purple to yellow. The acid pH activates the enzyme that causes decarboxylation of lysine to amines and the subsequent neutralization of the medium. This results in another color change from yellow back to purple. Bacteria that decarboxylate lysine turn the medium purple. In addition bacteria that produce H2S appear as black colonies.

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

3. Antibiotics susceptibility result for Bacteria isolates

Isolated bacteria	Antibiotics		AML	AMC	SXT	FOX	C	CTX	GE	TE	NOR	FN	OX	IMI	MEM
	Drug Susceptibility pattern	S													
		I													
		R													

NOTE: AML--Amoxycillin, AmC--Amoxycillin-Clavulanic acid, SXT----Sulphamethoxazol-trimethoperem, FOX----Cefoxitin, C---Chloramphenicol, CTX----Cefotaxime, GM--Gentamycin, TE--- Tetracycline, NOR---Norfloxacin, OX--- Oxacillin, IMI--- Imipenem, MEM--- Meropenem

Procedure for Performing the Disk Diffusion Test

Inoculum Preparation

- At least three to five well-isolated pure colonies of the same morphological type will be selected from Blood or MacConkey agar plate. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of tryptone soy broth.
- The turbidity of the broth culture will be adjusted with that of the 0.5 McFarland standards.

Inoculation of Test Plates

- Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level.
- The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface.

- The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Disks to Inoculated Agar Plates

- The predetermined battery of antimicrobial disks is dispensed onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure complete contact with the agar surface.
- The plates are inverted and placed in an incubator set to 37°C within 15 minutes after the disks are applied.

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate is examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disk. Zones are measured to the nearest whole millimeter, using sliding calipers which is held on the back of the inverted plate.

Annex II: English version of participant information sheet and consent form

2.1 Participant information sheet

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

Title: To determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from from March 30 to August 28/2015.

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

Postoperative wound infections are a major cause of mortality of patients in all age both developed and developing countries despite important progress in treatment and prevention of infectious diseases and use of modern technology. It is the most common infectious presentation in hospital acquired and community acquired infections since long time. Common etiologic agents of surgical site infections are S.aureus, E. coli, Klebsiella, Enterobacter, Citrobacter, Proteus, and Salmonella take the leading. In addition to their high prevalence the development of drug resistance is the challenge for controlling now days. As the frequency and antibiotic sensitivity pattern to common pathogen has been changing day by day, choice of appropriate antibiotics depends on the knowledge of common organisms and their antimicrobial susceptibility pattern in local scenario.

Hence the aim of this study is to determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from from March 30 to August 28/2015.

Aim of the study

The purpose of the study is determine To determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, results will be given to their physician for treatment or to get counseling. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

Risks and complication

There is no considerable risk(s) in participating in this study.

Confidentiality

In order to maintain the confidentiality of participants' information, the name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. No personal information will be 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.

Getachew Seyoum (PI): Signature: _____ Date: _____

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

PI Address: Getachew Seyoum: Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: getachewseyoum@yahoo.com; Tel.: +251911165939

1.1 Informed consent

I have been informed about the study which plans to determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from March 30 to August 28/2015.The objective and the application of the study were briefly explained to me. 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.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give pus for the mentioned study. 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 informed that results will be given to the Doctor who follow me 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 III: Amharic Version of the participant information sheet and Consent form (የተሳታፊዎች መረጃ ቅጽ ና የፈቃደኝነት ማረጋጫ)

1.1 የተሳታፊወች መረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮላጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

አርስት፡ በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች ለቀዶ ጥገነና ኢንፌክሽን በሽታ ከታከሙት ስርጭቱን እና ለመድሀኒት ያለዉን ግትርነት ማወቅ

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ።

መግቢያ

ለቀዶ ጥገነና ኢንፌክሽን በሽታ በተለያዩ ተህዋሲያን የሚመጣ የጤና ችግር ሲሆኑ በዓለም ላይ የሚያደርሱት የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም በታዳጊ ሀግሮች ውስጥ ቺግሩ ሰፊ ነዉ፡፡ ለዚህም ዋነኛ መንስኤው ብዙ አይነት ባክቴሪያዎች ሲሆን በሚያመጡት በሽታ ምክንያት የሚሞቱት ሰዎች ከፍተኛውን ቁጥር ይይዛሉ፤ በተጨማሪም እነዚህ ባክቴሪያዎች ለመድሐኒቶች በከፍተኛ ደረጃ የግትርነት ባህሪ(resistance) እያሳዩ መሆኑ ችግሩን ውስብስብ አድረጎታል፡፡ ሥለሆነም ይህን በሽታ ከፍተኛ ጉዳት ከማስከተሉ በፊት በመከላከል የሕብረተሰቡን ሞት ለመቀነስ ያለውን ስርጭት እንዲሁም ለመድሃኒቶችም ያለዉን የግትርነት ማዎቅ በጣም አስፈላጊ ነው፡፡

የጥናቱ አላማ

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች ለቀዶ ጥገነና ኢንፌክሽን በሽታ ከታከሙት ስርጭቱን እና ለመድሀኒት ያለዉን ግትርነት ማወቅ ደረጃ ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም። ነገር ግን ዉጤታቸዉ ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋና ተመራማሪው አድራሻ፤

- **ጌታቸዉ ስዩም፡** የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስ አበባ፣ ኢትዮጵያ
- **ኢ-ሜይል፣** getachewseyoum@yahoo.com
- **ስልክ ፣** +251911165939

1.2 የፈቃደኝነት ማረጋገጫ ቅፅ

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች ለቀዶ ጥገና ኢንፌክሽን በሽታ ከታከሙት ስርጭቱን እና ለመድሀኒት ያለዉን ግትርነት ማወቅ በሚል ርዕስ ለይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ የናሙናዉ ውጤት በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች ለተቋሞች እንደሚሰጥና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የደም ወይም የሽንት ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ____

Annex IV English version of Informed assent form for children between the ages of 12 – 15 Years

4.1 Participant information sheet

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

Title: To determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from from March 30 to August 28/2015.

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

Postoperative wound infections are a major cause of mortality of patients in all age both developed and developing countries despite important progress in treatment and prevention of infectious diseases and use of modern technology. It is the most common infectious presentation in hospital acquired and community acquired infections since long time. Common etiologic agents of surgical site infections are S.aureus, E. coli, Klebsiella, Enterobacter, Citrobacter, Proteus, and Salmonella take the leading. In addition to their high prevalence the development of drug resistance is the challenge for controlling now days. As the frequency and antibiotic sensitivity pattern to common pathogen has been changing day by day, choice of appropriate antibiotics depends on the knowledge of common organisms and their antimicrobial susceptibility pattern in local scenario.

Hence the aim of this study is To determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from from March 30 to August 28/2015.

Aim of the study

The purpose of the study is to determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from from March 30 to August 28/2015.

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, results will be given to their physician for treatment or to get counseling. Most importantly, this study will contribute to provide information or data for future and further nationwide study and to develop health programs for health policy makers.

I have checked with the child and they understand the benefits_____

Risks and complication

There is no considerable risk(s) in participating in this study.

I have checked with the child and they understand the risks and discomforts _____(initial)

Confidentiality

In order to maintain the confidentiality of participants' information, the name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. No personal information will be disclosed to third party or will not appear in any report from this study.

I have checked with the child and they understand the confidentiality of their information's and sample results _____(initial)

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.

Getachew Seyoum (PI): Signature: _____ Date: _____

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

PI Address: Getachew Seyoum: Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: getachewseyoum@yahoo.com; Tel.: +251911165939

4.2 Certificate of Assent

I have been informed about the study which plans to determine the prevalence of multidrug resistant bacteria in postoperative wound infection at Tikur Anbessa Specialized Hospital from from March 30 to August 28/2015.The objective and the application of the study were briefly explained to me. 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.

It is therefore with full understanding of the situation that I agreed to give the informed assent form voluntarily to the researcher to give pus for the mentioned study. 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 informed that results will be given to the Doctor who follow me and that I may ask the information if I want.

I have read this information (had the information read to me). I have had my questions answered and know that I can ask questions later if I have them.

Participant signature_____: Date: _____

Parent/Guardian has signed an informed consent yes_____No_____.

Annex V: Amharic Version of the participant information sheet and assent form for children between the ages of 12 – 15 Years (ከ 12-15 አመት ላሉ ህፃናት ተሳታፊዎች መረጃ ቅጽ ና የፈቃደኝነት ማረጋጫ)

5.1 የተሳታፊወች መረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮላጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

አርስት፡ በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች ለቀዶ ጥገና ኢንፌክሽን በሽታ ከታከሙት ስርጭቱን እና ለመድሀኒት ያለዉን ግትርነት ማወቅ

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል ያንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ።

መግቢያ

ለቀዶ ጥገና ኢንፌክሽን በሽታ በተለያዩ ተህዋስያን የሚመጣ የጤና ችግር ሲሆኑ በዓለም ላይ የሚያደርሱት የሞት እና የኢኮኖሚ ቀውስ ከፍተኛውን ደረጃ ይይዛል፡፡ በተለይም በታዳጊ ሀግሮች ውስጥ ቺግሩ ሰፊ ነዉ፡፡ ለዚህም ዋነኛ መንስኤው ብዙ አይነት ባክቴሪያዎች ሲሆን በሚያመጡት በሽታ ምክንያት የሚሞቱት ሰዎች ከፍተኛውን ቁጥር ይይዛሉ፤ በተጨማሪም እነዚህ ባክቴሪያዎች ለመድሐኒቶች በከፍተኛ ደረጃ የግትርነት ባህሪ(resistance) እያሳዩ መሆኑ ችግሩን ውስብስብ አድረጎታል፡፡ ሥለሆነም ይህን በሽታ ከፍተኛ ጉዳት ከማስከተሉ በፊት በመከላከል የሕብረተሰቡን ሞት ለመቀነስ ያለውን ስርጭት እንዲሁም ለመድሃኒቶችም ያለዉን የግትርነት ማዎቅ በጣም አስፈላጊ ነው፡፡

የጥናቱ አላማ

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች ለቀዶ ጥገና ኢንፌክሽን በሽታ ከታከሙት ስርጭቱን እና ለመድሀኒት ያለዉን ግትርነት ማወቅ ደረጃ ማሳየት፡፡

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም። ነገር ግን ዉጤታቸዉ ለሚከታተላቸዉ ሀኪም እንዲደርሰዉ ይደረጋል፡፡

ከጥናቱ የሚገኘዉ ጥቅም እንደሌሌ ህፃኑ እነደተረዳ አረጋግጫለሁ----------

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡

ከጥናቱ የሚደርስበት ጉዳት እና ተዛማጅ ችግር ዕንደሌሌ ህፃኑ እነደተረዳ አረጋግጫለሁ----------

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካልዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅ መሆኑ ህፃኑ እነደተረዳ አረጋግጫለሁ----------

የዋና ተመራማሪው አድራሻ፤

- **ጌታቸዉ ስዩም፡** የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ-አዲስ አበባ፣ ኢትዮጵያ
- **ኢ-ሜይል፣** getachewseyoum@yahoo.com
- **ስልክ ፣** +251911165939

5.2 **የፈቃደኝነት ማረጋገጫ ቅፅ**

በአዲስ አባባ ከተማ፣ በ ጥቁር አንበሳ ሆስፒታል ዉስጥ ባክቴሪያዎች ለቀዶ ጥገና ኢንፌክሽን በሽታ ከታከሙት ስርጭቱን እና ለመድሀኒት ያለዉን ግትርነት ማወቅ በሚል ርዕስ ለይ ለማጥናት በተመለከተ በሚደረገዉ ጥናት ላይ ለመሳተፍ መሆኑ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ የናሙናዉ ውጤት በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ዉስጥ አለመሳተፍ መብቴ እደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃደኝነቴን ሰጥቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች ለተቋሞች እንደሚሰጥና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በማገናዘብ ምርምሩ ላይ ስለኔ መረጃ እና የፐስ(pus) ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ____

በተጨማሪ ቤተሰብ የፈቃደኝነት ማረጋገጫ ሰጥተዋል