ISOLATION OF BACTERIAL PATHOGENS FROM PATIENTS WITH POSTOPERATIVE SURGICAL SITE INFECTIONS AND POSSIBLE SOURCES OF INFECTIONS AT UNIVERSITY OF GONDAR HOSPITAL, NORTHWEST ETHIOPIA

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LIST OF ABBREVIATIONS

BAP   Blood Agar Plate

CAP   Chocolate Agar Plate

CDC   Center for Disease Control and Prevention

C/S   Cesarean Section

HAIs   Hospital Acquired Infections

HCWs   Health Care Workers

ICU   Intensive Care Unit

MRSA   Methicillin Resistance *Staphylococcus aureus*

MSSA   Methicillin sensitive *Staphylococcus aureus*

QC   Quality Control

SOPs   Standard Operational Procedures

SPSS   Statistical Package for Social Sciences

SSI   Surgical Site Infection

VRE   Vancomycin Resistance *Enterococci*

ABSTRACT

Background: Hospital environment is a potential reservoir of bacterial pathogens since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible individuals. The increased frequency of bacterial pathogens in hospital environment is associated with a background rise in various types of nosocomial infections. Surgical site infection is one of the most frequent types of nosocomial infections in developing countries. The infection follows interference with the skin barrier, and is associated with the intensity of bacterial contamination of the wound at surgery or later in wards during wound care. Bacterial pathogens isolated from hospital environments are also known to develop resistance to multiple antimicrobial agents. The emergence of multi-drug resistant organisms in hospital results in difficulty to treat nosocomial infections.

Objective: The aim of this study was to isolate and identify bacterial pathogens from hospital environments & patients with postoperative surgical site infections and assess the antimicrobial susceptibility patterns of the isolates.

Methods: A cross sectional study was conducted at the University of Gondar Teaching Hospital from November 2010 - February 2011. In order to address the specified objectives, 220 specimens of pus, nasal, hand and surfaces swabs were collected using sterile cotton tipped swabs moistened with normal saline. Colony characteristics and Gram's technique were used to differentiate the organisms. Biochemical tests were done to confirm the species of the organisms. Antimicrobial sensitivity tests were done on the isolates using the disk diffusion method.

Result: A total of 268 bacterial pathogens were recovered from all specimens processed in the study. Most of the isolates, 142(52.9%) were from the environments. The rest, 77(28.8%) and 49(18.3%) were recovered from the health professionals and patients, respectively. The organisms associated with postoperative surgical site infections were S. *aureus* 11(22.4%) followed by *Klebsiella* species 10(20.4%) and *Proteus* species 9(18.4%), *Escherichia coli* 6(12.2%), *Enterobacter* species and coagulase negative staphylococci each 4(8.2%), *Pseudomonas aeruginosa* 3(6.1%) and *Citrobacter* species 2(4.1%). Gram negative rods isolated from different sample sources were deemed highly resistant to ampicillin 72(90%), cotrimoxazole, 68 (85%), doxycycline, 66 (82.5%), tetracycline, 63(78.8%), chloramphenicol, 48 (60%), nalidixic acid, 46 (57.5%) and gentamicin, 38 (47.5%). S. *aureus* demonstrated high level

of resistance to nalidixic acid and tetracycline while, ceftriaxone and ciprofloxacin were found to be relatively effective to all the isolates.

Conclusion: The predominant causes of postoperative surgical site infections were *S. aureus*, *Klebsiella* and *proteus* species. Medical equipment, environmental surfaces, air and hands of health personnel were found to be contaminated with various types of bacterial pathogens of nosocomial importance. It is imperative that all professionals should take an active role in infection control within their organization and more resources should be provided to encourage good antibiotic practice and good hygiene in the hospital.

Key words: Bacterial pathogen, postoperative surgical site infection, Hospital environments

1. INTRODUCTION

1.1. Background

Bacterial pathogens still play a considerable role in hospital acquired infections in Ethiopia. Nosocomial infections (also known as hospital associated/acquired infections) are those infections that develop in patients during their stay in hospitals or other type of clinical facilities, which were not present at the time of admission (Girard *et al*, 2002). The hospital environment is a potential reservoir of bacterial pathogens since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible/ immunocompromised individuals (Rhomberg *et al*, 2006). The increased frequency of bacterial pathogen in hospital environment is associated with a background rise in various types of nosocomial infections.

Surgical site infection (SSI) is one of the most frequent types of nosocomial infections in developing countries. The infection follows interference with the skin barrier, and is associated with the intensity of bacterial contamination of the wound at surgery or later in wards during wound care (Pryor *et al*, 2004). Wound infections have been a problem in the field of surgery for a long time. Previous studies from different parts of the Ethiopia showed that *S. aureus*, *Klebsiella species*, *Escherichia coli*, *proteus species, streptococcus species*, *Enterobacter species*, *pseudomonas species* and *coagulase negative staphylococci* were the most common pathogens isolated from wound (Biadglegne *et al*, 2009; Mulu *et al*, 2006). Rate of nosocomial infection are markedly higher in many developing countries, especially for infection that are largely preventable (e.g., those following surgical procedures). For instance the prevalence of post operative surgical site infection was reported as 44.1% of the patients with nosocomial infection from Mekelle, Ethiopia (Tesfahunegn *et al,* 2009).

Hospital acquired infections (HAIs) are largely preventable with implementation of effective control measures. The center for disease control and prevention (CDC) has pointed out that, "the most important measure for preventing the spread of nosocomial bacterial pathogens is effective hand washing". Most guideline recommends hand washing before and after contact with patients, before invasive procedure and after contact with contaminated inanimate objects (Garner *et al*, 1996).

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1.2. Statements of the problem

In countries where resources are limited, even basic life saving operations such as appendectomies and caesarean sections are associated with high infection rates and mortality. Consequently, on average having surgical site infection increases the patient's hospital stay by 7-10 days and leads to death (Tietjen *et al*, 1993). In elderly operative patients, surgical site infections were associated with almost four time's greater mortality, a mean attributable duration of hospitalization after surgery of 15.7 days and mean attributable hospital charges of $43, 970 (Keith *et al*, 2009). A study in United States of America suggested that programs that reduce the incidence of surgical site infections can substantially decrease morbidity and mortality and reduce the economic burden for patients and hospitals (Kirkland *et al*, 1999).

It is documented that, the source of postoperative surgical site infections can be either endogenous or exogenous. Patients may be infected by their body flora following surgical manipulation, chemotherapy and diagnostic or therapeutic procedures which in most cases suppress the natural body defense mechanisms (Pelczar *et al*, 1993). Exogenous, animate and inanimate sources of infections include hospital staff, other patients, and visitors, food, water, fomites, urinary catheter, intravenous devices, respiratory equipment and other prostheses (Jason *et al*, 2006; Prescott *et al*, 2005). Center for disease control and prevention (CDC) stated that contact transmission; direct from body surface to body surface or indirect transmission via contaminated inanimate objects within the hospital environments are some of the main routes of bacterial pathogens transmission (Garner *et al*, 1996; Manangan *et al*, 2001).

Bacterial pathogens that can able to survive in the hospital environment for long period of time and resist disinfection are particularly more important for nosocomial infections (Kramer *et al*, 2006). A systematic review of nosocomial pathogens indicated that most gram-positive bacteria, such as *Enterococcus* species including vancomycin resistant *Enterococci* (VRE), *Staphylococcus aureus* including methicillin resistance *Staphylococcus aureus* (MRSA) and *Streptococcus pyogenes* survive for months on dry surfaces. Many gram-negative bacteria, such as *Acinetobacter* species, *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, and *Serratia marcescens* can survive on inanimate surfaces even for months (Kramer *et al*, 2006).

Bacterial pathogens isolated from hospital environments are also known to develop resistance to multiple antimicrobial agents. The emergence of multi-drug resistance organisms in hospital resulted in difficulty to treat nosocomial infections. Examples of bacteria possessing such drug resistance are methicillin-resistant *S. aureus*, penicillin resistant pneumococci, vancomycin-resistant *Enterococci* and vancomycin resistant *S. aureus* (Prescott *et al*, 2005; Scheider-Linder *et al*, 2007).

Nowadays, the treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are often categorized according to their principal mechanism of action. Mechanisms include interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway, and disruption of bacterial membrane structure (Fred *et al*, 2006). It is documented that, Bacteria may be intrinsically resistant to more than one class of antimicrobial agents, or may acquire resistance by mutation or via the acquisition of resistance genes from other organisms. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to express efflux systems that prevent the drug from reaching its intracellular target, to modify the drug's target site, or to produce an alternative metabolic pathway that bypasses the action of the drug. Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons often facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids (McManus *et al*, 1997).

For example, *Staphylococcus aureus* has been reported as a major cause of community and hospital acquired infections. Infections caused by *S. aureus* used to respond to β -lactam and related group of antibiotics. However, due to development of methicillin resistance amongst *S. aureus* isolates (MRSA); treatment of these infections has become problematic. Indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse, and carriage of MRSA in nose are few important risk factors for MRSA acquisition (Kluytmans *et al*, 1997).

Despite the advance in modern medicine nosocomial infection still poses a risk of increased morbidity and mortality to patients. For this, the hospital environment may play a significant role. It is thereby important to identify environmental surfaces that are rich in bacteria and have the potential to harbor pathogens. Therefore, this study was undertaken to investigate the distribution and drug susceptibility pattern of potential bacterial pathogens isolated from patients with postoperative surgical site infection, health professionals and environmental samples in operating rooms and surgical wards at Gondar University Teaching Hospital.

2. LITERATURE REVIEW

2.1. Sources and transmission routes

Many factors are associated with HAIs, and a chain-of-infection model provides the best framework for depicting the relationships among these factors and SSIs. According to the chain-of infection model, a causative agent or pathogen survives within a reservoir, exits the reservoir via a mode of transmission, and enters a susceptible host; thereby causing disease. Intervention in any part of this process can stop disease transmission. Reservoir can be, soil, water, and inanimate surfaces. Of these, the most likely exogenous reservoir in the surgical setting is either an inanimate surface or a human (Wiley *et al*, 1979; APICE 1996). Hospital-acquired infections add to functional disability and emotional stress of the patient and may in some cases, leads to disabling conditions that reduce the quality of life. Nosocomial infections are also one of the leading causes of death. The economic costs are considerable. The increased length of stay for infected patients is the greatest contributor to cost (Girard *et al*, 2002; Kluytmans *et al*, 1997; Ponce-de-Leon *et al*, 1991; Plowman *et al*, 1999*)*.

2.2. Reviews on postoperative surgical site infections

A cross sectional study was carried out on drug sensitivity patterns of bacterial isolates from septic postoperative wounds in Jinja hospital, Uganda. Pathogenic bacteria were recovered from 58.5% of the specimens. The isolates were: *S. aureus* 45.1%, Coliforms 16.9%, *Proteus mirabilis* 11.3%, *P. aeruginosa* 9.9%, *Klebsiella pneumoniae* 7.0% and *Enterobacter* species 2.82% (Anguzu *et al*, 2007).

A study was carried out to determine the prevalence of different pathogens in surgical wounds. 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%, gentamicin 60%, streptomycin 60%. Resistance to beta-lactam antibiotics was common among gram negative bacteria. Some isolates of *Pseudomonas aeruginosa* were resistant to Gentamicin 18.70% and Streptomycin 35.70 % (Nwachukwu *et al*, 2009).

Another survey was conducted in logos Nigeria, to determine the prevalence of *Pseudomonas aeruginosa* 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 aeruginosa*, 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 pyogenes* 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 (Oguntibeju *et al*, 2004).

A prospective study was done in Addis Ababa to determine the magnitude of nosocomial infections and isolate the bacterial etiologic gents in a tertiary hospital. Two hundred fifteen patients admitted in surgical ward and intensive care unit were included in the study. Of these patients 35.8% of them developed different forms of nosocomial infections. Surgical site infection comprised of 23(29.8%). Bacterial pathogens identified as cause for SSI were *Escherichia coli* 11.4%, P. *aeruginosa* 22.7%, *K. pneumoniae* 15.9%, *P. vulgaris* 13.6%, *E. cloacae* 2.3%, *K. oxytoca* 4.5%, C. *braakii* 4.5%, S. *aureus* 15.9% and *CNS* 9.1% (Endalafer *et al*, 2011).

A laboratory based retrospective study of wound swabs was conducted in the microbiology department at Bahir Dar Regional Health Research Laboratory. From the total of 379 wound swabs, bacterial isolates were found on 201 patients with an isolation rate of 53.0%. *Staphylococcus aureus* was the predominant isolate 140 (69.7%) followed by *proteus species* 19 (9.5%) and *Klebsiella species* 10 (5.0%). The overall multiple drug resistance patterns in ten antibiotics was 97.5% (Biadglegne *et al*, 2009).

Another laboratory based retrospective study of 151 wound swabs was conducted in Gondar University Teaching Hospital. In the study bacterial pathogens were isolated from 79 wound swabs. *Staphylococcus aureus* was the predominant isolate 51 (65%) followed by *Escherichia coli* 8(10%) *Klebsiella* species 7(9%), *Proteus* species 3(4%) and the overall multiple drug resistance patterns in ten antibiotics was 78.5%. Single and multiple drug resistance to the commonly used antibiotics were very high among bacterial isolates from wound (Mulu *et al*, 2006).

2.3. Reviews on contamination of hospital environments

Different studies in various parts of the world had assessed the extents of bacterial contamination of hospital environments. For example, a cross sectional study to analyze the distribution of probable nosocomial pathogens in a government hospital in Nigeria was conducted. Samples were obtained from doctors, nurses, orderlies, patients, air, and fomites like beds, cannula, oral thermometer, and table. A total of 56 bacteria were isolated. Gram positive cocci were the highest number of isolates of which *Staphylococcus epidermidis* (22; 39.2%), *Staphylococcus aureus* (16; 28.5%) and *Streptococcus* spp. (5; 8.9%). Among the Gram negative bacilli, *Escherichia coli* were the highest (4; 7.1%). Others were *Klebsiella pneumoniae* (3; 5.3%), *Proteus* spp. (2; 3.5%) and *Enterobacter aerogenes* (2; 3.5%). Orthopedic ward (22 isolates) had the highest number of isolates followed by pediatric ward (15 isolates). Surgical and medical wards had 10 and 9 isolates, respectively (Chikere *et al*, 2008).

Another study aimed to investigate the hygienic conditions of air at delivery and nursing rooms in three hospitals in Khartoum was also conducted. Seventy nine samples from delivery room and 60 samples from nursing rooms were collected, while 63.3% air samples from delivery and 66.7% from nursing rooms were positive for bacterial growth. The isolated species were identified as *S. aureus, Escherichia coli, Klebsiella* species and *P. aeruginosa. Staphylococcus aureus* and *P. aeruginosa* were the most dominant organisms isolated from the delivery rooms at all examined hospitals, while *S. aureus* showed the highest percentage from nursing rooms at two of the examined hospitals (Sana *et al*, 2010).

A cross sectional study to determine the extent of contaminations of patient's medical file in Taiwan demonstrated the following. Ninety percent of charts in surgical ward and 72% in ICU were contaminated with bacteria pathogens. Coagulase negative staphylococcus was the predominant isolate in both surgical ICU 44% and surgical ward 53.3%. Other bacterial isolates were *Klebsiella* species, *Acinetobacter species*. In the study it had be concluded that that patients char may be the source for cross infection in surgical unites (Sing-on *et al*, 2009).

A study aimed to identify the nosocomial bacteria commonly found on x-ray equipment and accessories and assess the effectiveness of some common chemical disinfectants used in x-ray units. Bacterial agents were isolated in 142 swabs representing 47.2% of all the swab samples. *Staphylococcus aureus*, *Klebsiella* species, Coliforms and *Staphylococcus epidermidis* were the bacteria isolated from the swab samples. *Klebsiella* species were isolated most often (49 times; 34.5%) and *staphylococcus epidermidis* were isolated the least number of times (18 times; 12.7%). The x-ray cassettes recorded the highest number of times bacteria were isolated (54 times; 38%) with Coliform*s* being isolated most often (45 times; 31.7%) (Ochie *et al*, 2009).

A study conducted in An-Najah University Hospital operating room demonstrated contamination of various inanimate objects with potential pathogenic bacteria. *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococci* were isolated from saline solution kept in glass bottles for washing and cleansing wounds, suction machines, respirators, endotracheal tubing, oxygen pumps and sinks. *Alcaligenes odorans* was isolated from the suction machines. *Aeromonas species were* isolated from deionized water and sinks, the implications of these findings were also discussed as the hospital environment becomes the source of hospital acquired infection. Proper disinfection of equipments was suggested as solution in this study (Yahya *et al*, 1995).

2.4. Reviews on health professionals

A one year prospective study to ascertain the prevalence of nasal carriage of potentially pathogenic bacteria in health care workers and the antibiotic susceptibility profile were conducted in Pakistan. The prevalence of *Staphylococcus aureus*, coagulase negative staphylococci and methicillin resistant *Staphylococcus aureus* were 48%, 46% and 14% respectively. The most effective antibiotic for *S. aureus* was found to be vancomycin with 100%

efficacy, then cephalothin 92%, ciprofloxacin 91%, amikacin 77%, erythromycin 55%, ampicillin 11% and penicillin 3%. Coagulase negative staphylococci were 100% sensitive to vancomycin and cephalothin. Oxacillin showed 78% effectiveness, while ampicillin and penicillin, demonstrated 64% and 59% respectively. Doxycycline 93%, amikacin 93%, fusidic acid 90% and erythromycin 92% were effective antimicrobials (Kalsoom *et al*, 2008).

The contamination rates of Health Care Worker's (HCW) mobile phones and resistance to commonly used antimicrobials were evaluated in three teaching hospitals in Kerman, Iran. One hundred fifty swab specimens were taken from HCWs dominant hand and their mobile phone. A total of 48 (32.0%) mobile phones and 59(39.3%) of dominant hands had bacterial contamination and *Staphylococcus epidermidis* was the most commonly cultured organisms from all sites. The resistance rates to commonly used antimicrobials in isolated bacteria from phones and dominant hand varied from 6.7% for cephalothin to 25% for amoxicillin, respectively. Therefore, mobile phones and hands of health care workers could be an important source of nosocomial infections and the spread of bacterial resistance bacteria in medical healthcare settings (Gholamreza *et al*, 2009).

3. SIGNIFICANCE OF THE STUDY

This study was carried out to gain insight into the distribution and carriage rate of pathogenic bacterial species that could be of potential health risk in a hospital or any other healthcare facility. The study assesses the distribution of bacterial pathogens in patients, health professionals and hospital environment. Since the bacterial pathogens that predominate in particular surgical units often change in relation to newly admitted patients and altered therapy protocols, knowledge of the bacterial agents that are generally a problem in surgical units might result in wrong selection of empirical systemic antibiotics. It becomes therefore essential for the surgical units at university of Gondar Hospital to determine the profile of surgical site wound colonization and antimicrobial resistance profiles.

Evidence based knowledge about the extent of contamination of the hospital environment is important for designing and implementing effective prevention and control measure to tackle postoperative surgical site infections and other forms of hospital acquired infections. Moreover the study finding may give an insight for health professionals in University of Gondar teaching Hospital to take the utmost care for their patients by breaking the chains of transmission.

The current study also plays a great role in describing antimicrobial susceptibility pattern of isolates to the common antibiotics used in the area. Hence, the study finding is important in setups where immediate culture and sensitivity tests are difficult, sound epidemiological knowledge of bacterial pathogens helps in rationale selection of antibiotics for prophylaxis and empiric treatment options. Thus, morbidity and mortality associated with infections by bacterial agents provide a strong argument for our intention to identify possible bacterial pathogens from patients, health professionals and environment thereby implementing strict rules to control their spread.

4. OBJECTIVES OF THE STUDY

4.1. General Objective

- To assess the distribution of potential bacteria pathogens in patients with postoperative surgical site infection, health personnel & hospital environments

4.2. Specific Objectives

- To identify the predominant cause of postoperative surgical site infections in admitted patients.
- To identify common bacterial pathogens contaminating the environment of operating room and surgical wards.
- To analyze *S. aureus* nasal carriage rate and bacterial contamination of hands of health professionals at surgical units.
- To assess the antimicrobial susceptibility pattern of bacterial isolates.

5. MATERIALS AND METHODS

5.1. Study area and period

The study was conducted at Gondar University Teaching Hospital which is located in Gondar town in Amhara regional state. Gondar is 739 km far from Addis Ababa to the northwest of Ethiopia. The University Hospital is one of the biggest tertiary level referral and teaching hospitals in the region. A large number of people from the surrounding zones and nearby regions visit the hospital both for inpatient and as an outpatient treatment. This teaching hospital consists of an operating room, intensive care unit (ICU) with 12 beds, 13 wards with 327 beds, and outpatient departments. The study was carried out from November 2010 - February 2011.

5.2. Study design

A hospital based cross sectional study was conducted. Information, clinical sample and environmental samples which were relevant to the study were collected from the study populations.

5.3. Source population

All the patients who had undergone operation and admitted; all health personnel and all inanimate objects which were found in the operating room and surgical ward were the source population of the study.

5.4. Study population

All patients who had developed postoperative surgical site infection (septic wound) during the study period, all staff in operating room and surgical wards and all inanimate objects suspected to harbor bacterial pathogens in operating room and surgical ward were the study populations.

5.5.   Sample size and sampling technique

The sampling technique was convenient. All the 42 patients who had developed postoperative surgical site infection during the study period were included in the study. Thirty six (36) volunteer health professionals in operating room, surgical and orthopedic wards were also included. In addition, a total of 142 inanimate objects within the surgical units and that could be touched with hands of health professionals, patients or attendants were screened for bacterial contamination.

5.6.   Exclusion criteria

Patients who do not develop postoperative surgical site infection on clinical examination during the study period were excluded from the study.

5.7.   Definition of Terms

Surgical site infection: a type of healthcare-associated infection in which a wound infection occurs at site of surgery. The diagnosis was based on the following criteria: pus, serous or non purulent discharge from surgical site, signs of inflammation (oedema, redness, heat, fever, indurations and tenderness). Operations can be classified as Clean- in which no inflammation is encountered and the, respiratory, alimentary or genitourinary tracts are not entered. Clean-contaminated- in which the respiratory, alimentary or genitourinary tracts are entered but without significant spillage. Contaminated - where acute inflammation is encountered, or there is visible contamination of the wound. Dirty in the presence of pus, where there is a previously perforated hollow viscous or compound/open injury more than four hours old (Garner JS ,1996).
Environmental samples: swab specimens that were taken from inanimate objects and air in surgical units of the Hospital.
Surgical unit is to mean operating room, surgical and orthopedic wards.

5.8.   Variables of the Study

Independent variables: Age and sex of patients, medical devices, various surfaces and inanimate objects such as sink, floor, surfaces, bed frames, walls, equipments, air.
Dependant variables: bacterial isolates, drug susceptibility pattern (susceptible, resistance, intermediate)

5.9. Data Collection and processing

5.9.1. Specimen collection.

Wound swabs: From all the 42 patients whose diagnosis was confirmed as wound sepsis by a surgeon, swabs of wound secretions were aseptically obtained from surgical sites before the wound was cleaned with an antiseptic solution and before antibiotic therapy is started. Specimens were collected on sterile cotton swabs without contaminating them with skin commensals.

Nasal and hand swab: Nasal and hand swabs were taken from 36 health professionals. A sterile cotton swab moistened with normal saline was passed into the anterior nares of both the nostrils and rotated in both directions. A separate sterile cotton swab was rotated on the palms, fingers and finger nails of the dominant hand health professionals.

Environmental sample: one hundred forty two environmental specimens were collected from medical devices(such as suction machine, operating table, oxygen cylinder, blood pressure apparatus, light source, sterile materials) , air, and inanimate objects (such as floor areas, walls, bed-frames, door handles, light switches, sinks, stands for infusion apparatus and disinfectants). Sterile cotton tipped swabs moistened with normal saline was rotated against the surface of inanimate objects to obtain specimens. For air samples, blood agar plates were distributed at various distance in the operating room and wards and left opened to the air for 1hour (Sana *et al*, 2010). Soon after collection samples were transported to the microbiology laboratory.

Transportation of specimens: following collection from patients, health professionals and environments specimens were transported by placing each swab in a separate sterile test tube to the microbiology laboratory within 30 minutes.

5.9.2. Sample Processing

Following collection, the swabs were inoculated into MacConkey agar, blood agar plates (BAP) and manitol salt agar (Oxoid, LTD). The inoculated agar plate was incubated at 35°C for 24-48 hours. Then the growth was inspected to identify the bacteria.

Preliminary identification: Presumptive identification of bacteria were based on gram reaction, colony characteristics of the organisms like haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms (Elmer *et al*, 1997).

Biochemical tests: Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests (Oxoid, LTD). Namely, triple sugar iron agar, indole, Simon's citrate agar, lysine iron agar, urea, manitol and motility. Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results (Cheesbrough *et al*, 2000).

Susceptibility testing: Susceptibility testing was performed on isolates based on the agar disc-diffusion technique developed by Bauer *et al* (1966). The suspension of the test organism were prepared by picking parts of similar test organisms with a sterile wire loop, suspended in sterile broth and incubated up to two hours to allow organisms reach their log-phase in growth. The densities of suspension to be inoculated were determined by comparison with opacity standard on McFarland 0.5 Barium sulfate solution (Bauer *et al*, 1966*)*. A sterile swab was dipped into the suspension of the isolate in broth, squeezed free from excess fluid against the side of bottle. The test organism were uniformly seeded over the Mueller-Hinton agar surface (Oxoid, LTD) and exposed to a concentration gradient of antibiotic diffusing from antibiotic impregnated paper disk into the agar medium. The medium was then incubated at $35^{\text{o}}C$ for 18-24 hours. Grades of susceptibility pattern were recognized as sensitive and resistant by comparison of zone of inhibition as indicated in the manufacturer's guide. The drugs tested for both gram negative and gram positive bacteria were ampicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), tetracycline (µg), cotrimoxazole (25µg) chloramphenicol (30µg), doxycycline (30µg), nalidixic acid (15µg) and ceftriaxone (30µg). Methicillin (5µg), penicillin (10IU), erythromycin (15µg) and vancomycin (30µg) were used for only gram positive bacterial isolates.

5.10. Quality Control

The reliability of the study findings was guaranteed by implementing Quality control (QC) measures throughout the whole processes of the laboratory works. All materials, equipment and procedures were adequately controlled. Culture media were tested for sterility and performance. Pre-analytical, analytical and post-analytical stages of quality assurance that are incorporated in standard operating procedures (SOPs) of the microbiology laboratory of Gondar University were strictly followed. International Control bacteria strains**:** *Escherichia coli* (ATCC 25922) S.

aureus (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used in controlling the tests carried out in this study. To standardize the inoculum density of bacterial suspension for the susceptibility test, a barium sulfate (BaSO4) turbidity standard, equivalent to a 0.5 McFarland standard was used (Cheesbrough *et al*; 2000, Bauer *et al*, 1966).

5.11. Data Analysis

The data was analyzed using SPSS statistical software version 17. Then, study findings were explained in words and tables. Proportions for categorical variables were compared using chi-square test. In all cases P-value less than 0.05 was taken as statistically significant.

5.12. Ethical Considerations

The proposal was approved by the Department ethical review committee of Microbiology Immunology and Parasitology, School of Medicine, Addis Ababa University. Permission was also obtained from the Gondar University Hospital administrator. Subjects were recruited after they become informed about the objectives and use of the study. Subjects had full right to continue or withdraw from the study. For each confirmed infections the responsible clinician of the subjects was informed and treatment was started as per the guideline in the hospital. Information obtained at each course of the study was kept confidential.

6. RESULTS

6.1. Bacterial isolates of patients, health professionals and environments

A total of 220 swab specimens were collected from patients, health professionals and hospital environments. Of these, 42(19.1%) were from patients with postoperative surgical site infection, 142 (64.5%) from inanimate objects in the hospital and 36(16.4%) from health professionals. A total of 268 bacterial pathogens were recovered from all specimens processed during the study. Of these, 70.1% (n =188) were gram-positive and 29.9% (n = 80) were gram-negative bacteria. Forty three out of the 220 swabs (19.5 %) had mixed growth, while 144 (65.5 %) had pure (single) bacterial growth. The rest, 33 (15 %) had no bacterial growth. Majority of the bacteria, 142(53%) were isolated from the environment. The rest, 77(28.7%) and 49(18.3%) were recovered from health professionals and patients, respectively. Among the gram positive isolates, coagulase negative staphylococci were predominant from the health professionals and the environments followed by *S. aureus. Klebsiella* specie*, Escherichia coli*, *Proteus* specie*s* and *Pseudomonas aeruginosa* were the most common isolate of the gram negative rods (Table 1).

Table 1. Bacterial pathogens isolated from environmental samples, patients and health professionals of surgical units at Gondar University Hospital, November - February 2011

Bacterial isolate	Environment No (%)	Patients No (%)	Health professionals No (%)	Total
Gram positive	**101(71.1)**	**15(30.6)**	**72(93.5)**	**188(70.1%)**
CN Staphylococcus	69 (68.3)	4(26.7)	44(61.1)	117(62.2)
Staphylococcus aureus	31(30.6)	11(73.3)	28(38.9)	70(37.3)
Enterococcus species	1(0.99)	0(0)	0(0)	1(0.5)
Gram negative	**41(28.9)**	**34(69.4)**	**5(6.5)**	**80(29.9%)**
Escherichia coli	10 (24.4)	6(17.6)	1(20)	17(21.3)
Klebsiella species	11(26.8)	10(66.7)	3(60)	24(30)
Pseudomonas aeruginosa	8(19.5)	3(20)	0(0)	11(13.8)
Enterobacter species	4(0.9)	4(26.7)	1(20)	9(11.2)
Citrobacter species	2(0.5)	2(13.3)	0(0)	4(5)
Proteus species	5(12.2)	9(60)	0(0)	14(17.5)
Serratia specie*s*	1(2.4)	0(0)	0(0)	1(1.2)
Total	142(53)	49(18.3)	77(28.7)	268(100)

6.2.   Postoperative Surgical site infections

A total of forty two (42) patients presenting with postoperative septic surgical site infection during clinical examination were enrolled in the study. Out of 42 patients studied, 27(64.3%) were males and 15(35.7%) females. The age of study groups ranged from 4 to 77 year, with a median age of 29 year. The preoperative stay in the hospital ranged from 1 to 32 days. Majority of the patients, 32(76.2 %) had stayed in the hospital for 1 to 15 days before operation.

Majority of the study participants, 39(92.9%) were given antibiotics prophylaxis before surgical operation, while 3(7.1%) were not given any antibiotics. The prophylaxis used were combination of ampicillin/gentamicin/chloramphenicol 20(51.3%), ampicillin 13(33.3%) and gentamicin 6 (15.4%).

Among the various surgical procedures performed during the study period, postoperative surgical site infections were observed from patients who had Laparatomy 13 (31%), caesarean section 6(14.3%), prostatectomy 7(16.7%) appendectomy 3(7.1%), thyroidectomy 3(7.1%) and others 5(12%) as shown in the table 3.

The majority of wound swabs, 37(88.1%) had bacterial growth within 18-24 hours of incubation. Twelve out of 37 (32.4%) had mixed growth, while 25 (67.6%) had pure bacterial growth. The rest, 5(11.9%) had no bacterial growth even after 48 hours of incubation. The rate of bacterial isolation among those patients who had clinically septic wound infections was 88.1%. The number of bacterial isolates from males 25 (59.5 %) were higher than females 12(28.5%). The difference is not statistically significant (p=0.227). Thirty four (69.4%) of the isolates of postoperative surgical site infections were gram-negative bacteria. *Klebsiella* species 10(20.4 %) were the predominant isolate followed by *Proteus* species 9(18.4%), *Escherichia coli* 6(12.2%), *Enterobacter* species 4(8.2%), P*seudomonas aeruginosa* 3(6.1%) and *Citrobacter* species 2(4.1%). Fifteen (30.6%) of the isolates were gram-positive cocci. Of these, *S. aureus* accounted for 11(22.4%) and coagulase negative staphylococcus 4(8.2%) of the total bacterial isolates of wound swabs.

Table 2. Profiles of bacterial isolates identified in postoperative wound infection Gondar University Hospital, November 2010 - February 2011

Bacterial isolate	Total	Percentage
Gram positive	**15**	**30.6**
Staphylococcus aureus	11	22.4
Coagulase negative staphylococcus	4	8.2
Gram negative	**34**	**69.4**
Klebsiella species	10	20.4
Proteus species	9	18.4
Escherichia coli	6	12.2
Enterobacter species	4	8.2
Pseudomonas aeruginosa	3	6.1
Citrobacter species	2	4.1
Total	**49**	**100**

Most of the isolate, 22(44.9%) were recovered from patients who had Laparatomy. Of these, gram negative bacteria accounted for 19(86.4%) of the isolates. The predominant isolate from Laparatomy was *Proteus* species 6(27.2%) followed by *Klebsiella* species 4(18.2%), *Enterobacter* speci*es* and *Pseudomonas aeruginosa* each 3(13.6%) and C*itrobacter* species 1(4.5%). The rest, three (13.6%) of the isolates were *S. aureus* from gram positives. Eight (88.9%) of the isolates from patients who had prostatectomy was gram negatives. *Klebsiella* species 4 (44.4%), *Escherichia coli* 2(22.2%) and *Proteus* species 1(11.1%) were the common isolates. Only coagulase negative staphylococcus 1(11.1%) was isolated among the gram positives. The predominant isolate in amputation and thyroidectomy were *S. aureus* (Table 3).

Table 3. List of surgical procedures and corresponding bacterial isolates from patients who had post operative surgical site infection, November 2010 - February 2011

Site of surgical procedures	Patients No (%)	Bacterial isolates		Total isolates No (%)
		Gram positive No (%)	Gram negative No (%)	
Laparatomy	13(30.9)	3(13.6)	19(86.4)	22(100)
Prosteoctomy	7(16.7)	1(11.1)	8(89.1)	9(100)
Cesarean section	7(16.7)	2(33.3)	4(66.7)	6(100)
Amputation	5(11.9)	3(60)	2(40)	5(100)
Thyroidectomy	3(7.1)	2(66.7)	1(33.3)	3(100)
Appendectomy	3(7.1)	1(50)	1(50)	2(100)
Excision	1(2.4)	0(0)	0(0)	0(0)
Fixation	1(2.4)	0(0)	1(100)	1(100)
Debridement	1(2.4)	1(100)	0(0)	1(100)
Incision	1(2.4)	0(0)	0(0)	0(0)
Total	42(100)	15(30.6)	34(69.4)	49(100)

Note: gram negative isolates were *Escherichia coli, Klebsiella* species, *Enterobacter* species, *Citrobacter* species *pseudomonas aeruginosa* and *Proteus* species. Whereas gram positive isolates were *S. aureus* and Coagulase negative staphylococci.

6.3. Environmental survey of bacterial pathogens

In this study142 swab specimens were collected from various environmental sources 56 located in the operating room, 42 in surgical and 44 in orthopedic wards (Table 4). From these, 118 (83.1%) of the inanimate objects; 48 (33.8%) in operating room, 36(25.4%) in surgical ward and 34 (23.9%) in orthopedic wards had demonstrated evidence of bacterial contamination. Twenty six out of 118 (22.0%) inanimate objects had mixed bacterial growth, while 92(77.9%) had pure growth. Twenty four (16.9%) of the inanimate objects; 8(5.7%) in operating room, 6 (4.2%) in surgical ward and 10(7.0%) in orthopedic ward did not show any bacteria growth even after 24 hours of incubation. Out of one hundred forty two bacterial pathogens isolated from inanimate objects 101 (71.1%) and 41 (28.9%) were gram positive and gram negative, respectively.

The hospital environment that had the highest number of bacterial isolates was the operating rooms. These rooms accounted for 52 out of the 142(38%) isolates from all the surgical units. Surgical ward followed closely with 48/142(33.8%) isolate, while orthopedic ward had 42/142(29.6%). The most commonly isolated gram positive bacteria from the surgical units were coagulase negative staphylococci 69(68.3%) followed by *S. aureus* 31(30.7%) and *Enterococcus* species, 1(1%). Similarly, *Klebsiella* species 11(26.8%), *Escherichia coli* 10(24.3%), *Pseudomonas aeruginosa* 8(19.5%), *Proteus* species 5(12.2%) and *Enterobacter* 4 (9.8%) were common among gram-negative isolates. Citrobacter 2(4.9%), *Serratia* and *Enterococcus* species each 1(2.4%) were the least isolated.

According to the statistical analysis and calculated p-values, surgical units ($x^2 = 1.54$, P= 0.465) and moisture content of the objects ($x^2=3.41$, P=0.065) did not show statistically significance association with bacterial contamination of inanimate objects.

Table 4. The distribution pattern of bacterial isolates in different surgical units of Gondar University Hospital, November 2010 - February 2011.

Bacterial isolate	Surgical units			Total
	Orthopedic ward	Surgical ward	Operating room	
Gram negative	**13(30.9)**	**15(31.3)**	**13(24.1)**	**41(28.9)**
Escherichia coli	2(15.3)	3(20)	5(38.4)	10(24.3)
Klebsiella species	3(23.1)	5(33.2)	3(23.1)	11(26.8)
Enterobacter species	3(23.1)	1(6.7)	0(0)	4(9.8)
Citrobacter species	1(7.7)	0(0)	1(7.7)	2(4.9)
P. aeruginosa	3(23.1)	4(26.7)	1(7.7)	8(19.5)
Serratia species	0(0)	1(6.7)	0(0)	1(2.4)
Proteus species	1(7.7)	1(6.7)	3(23.1)	5(12.2)
Gram positive	**29(69)**	**33(68.7)**	**39(75)**	**101(71.1)**
Staphylococcus. aureus	7(24.1)	12(36.3)	12(70.8)	31(30.7)
CN staphylococcus*	21(72.4)	21(63.6)	27(69.2)	69(68.3)
Enterococcus species	1(3.4)	0(0)	0(0)	1(1)
Total	**42(29.6)**	**48(33.8)**	**52(38)**	**142(100)**

* CNS- Coagulase negative staphylococcus

Gram positive bacteria in the genus *staphylococci* were found contaminating most dry surfaces. Whereas, gram negative rods especially in the family of Enterobacteriaceae were most frequently isolated on moisten objects such as sink (Table 4). A total of 23 bacterial pathogens were isolated from sinks. Of these, 22(95.6 %) and 1(4.4%) were gram negative and gram positive, respectively. From the total isolates of sinks *Escherichia coli* 6(27.3%), *Klebsiella* species 5 (27.7%), *Pseudomonas aeruginosa* 4(18.2%), *Enterobacter*, *Citrobacter* and *proteus* species each 2(9%) and *Serratia* species 1(4.5%) were the common isolates. Other inanimate objects such as floor, tables, door handles, light switch, infusion sands, bed- frames and medical equipment were found to be contaminated with gram positive bacteria of the genus staphylococcus. Fortunately, Bacterial pathogens were not identified from antiseptic solutions and sterile materials such as forceps and scissors.

A total of 22 bacterial pathogens were recovered from air samples of surgical units of the hospital. Eighteen (81.8%) and 4(18.2 %) were gram positive and gram negative, respectively. The predominant isolate in the air sample was coagulase negative staphylococci 12(53.6%) followed by *S. aureus* 6(27.3%), *Pseudomonas aeruginosa* 2(9.1%), *Proteus* species and *Enterobacter* species each 1(4.5%) (Table 5).

Table 5. Analysis of swabs of inanimate objects for the presence of bacterial pathogens at Gondar University Hospital, November 2010 - February 2011

Objects screened	Number	Bacterial isolates		Total
		Gram positive	Gram negative	
Floor	10	8	4	12
Table surface	12	11	2	13
Wall	6	3	0	3
Door handle	14	12	3	15
Light switch	10	9	2	11
Air	21	18	4	22
Sink	16	1	22	23
Bed frame	14	14	3	17
Oxygen cylinder	4	3	0	3
Operating table	4	4	0	4
Suction machine	4	4	0	4
BP apparatus	4	3	0	3
Light source	4	3	0	3
Infusion stands	12	8	1	9
Disinfectant	3	0	0	0
Sterile objects	4	0	0	0
Total	142	101	41	142

Note: gram negative isolates were *Escherichia coli, Klebsiella* species*, Enterobacter* species, *Citrobacter* species *pseudomonas aeruginosa, Serratia* species *and Proteus* species. Whereas gram positive isolates were *S. aureus. Enterococcus* species and Coagulase negative staphylococcus

6.4. Hands and nasal carriage of health professionals

Out of 40 health professionals approached, 36 agreed to participate. This comprises 24 (66.7%) males and 12(33.3%) females with ages ranging from 22 to 50 years (mean age 32.4 year). Seventy two swab specimens were collected from the dominant hand and nostrils of health professionals at Gondar University Teaching Hospital. A total of 77 bacterial pathogens of nosocomial importance were isolated. Of these, 36(46.8%) and 41(53.2%) were from nostril and hands, respectively. Seventy two of the isolates were gram positive, while 5(6.5%) were gram negatives. Coagulase negative staphylococcus 44(57.1%) was the predominant isolate followed by *S. aureus* 28 (36.4%).

Thirty two (88.9%) of the health professionals hand were found to be contaminated with one or more bacterial pathogens of nosocomial importance. Coagulase negative staphylococci 23(56.1%), *Klebsiella* species 3(7.3%), *Escherichia coli* and *Enterobacter* species 1(2.4%) each were isolated from the dominant hands of health professionals. From 8 (22.2%) of the health professionals *S. aureus* were isolated from both of their hand and nares. In two of them, S. *aureus* isolated from hand and nares had identical antibiogram pattern.

Table 6. Common bacterial isolates from the dominant hand and nostrils of health professionals at Gondar University Hospital, November 2010 - February 2011

Bacterial isolates	Hand swabs No (%)	Nasal swabs No (%)	Total No (%)
Gram positive	**36(87.8)**	**36(100)**	**72(93.5)**
Coagulase negative staphylococcus	23(88.4)	21(58.3)	44(57.1)
Staphylococcus aureus	13(11.6)	15(41.7)	28(36.4)
Gram negative	**5(12.2)**	-	**4(5.2)**
Klebsiella species	3(7.3)	-	3(3.9)
Escherichia coli	1(2.4)	-	1(1.3)
Enterobacter species	1(2.4)	-	1(1.3)
Total	41(53.2)	36(46.8)	77(100)

6.5.   Antimicrobial susceptibility test

The result of antimicrobial susceptibility pattern of the isolate is shown on table 7 below. In general, Gram negative rods isolated from different sample sources were deemed highly resistant to most of the antibiotics tested. Of the isolates, 72(90%), 68(85%), 66(82.5%), 63(78.8%), 48(60%), 46(57.5%) and 38(47.5%) were found to be resistant to ampicillin, cotrimoxazole, doxycycline, tetracycline, chloramphenicol, nalidixic acid and gentamicin in their respective order. Among other antibiotics ceftriaxone 48(40%) and ciprofloxacin 61(76.3%) were relatively effective against gram- negative bacterial isolates.

Among the gram negatives, the predominant isolate *Klebsiella* specie demonstrated high level of resistance to ampicillin 22(91.7), cotrimoxazole, gentamicin, chloramphenicol and doxycycline each 20(83.3%), tetracycline 19(79.2%), ceftriaxone and nalidixic acid each 16(66.7%). Relatively ciprofloxacin were effective against 15(62.5%) of the *Klebsiella* species.

 More than 80% of *Escherichia coli* isolates were resistance to tetracycline, ampicillin, cotrimoxazole, and doxycycline. Ciprofloxacin 12(70.6%) and ceftriaxone 14(82%) were effective against *Escherichia coli*. 10 out of 11(90. 9%) isolates of *Pseudomonas aeruginosa* demonstrated high level of resistance to tetracycline, ampicillin, cotrimoxazole, nalidixic acid, doxycycline and chloramphenicol. Three (75%) of *Citrobacter* species isolates were resistance to all the antibiotics tested with the exception of 2(50%) susceptibility to ciprofloxacin. Twelve (85.7%) of *Proteus* species were found to be resistant to chloramphenicol, ampicillin and ciprofloxacin. Gentamicin and tetracycline each 12(85.7%), ceftriaxone, cotrimoxazole, doxycycline and nalidixic acid 8(57.1%) were effective against *Proteus* species.

Enterobacter isolates were also highly resistance to most of the antibiotics tested: cotrimoxazole, ampicillin and chloramphenicol each 7(77.8%), doxycycline and nalidixic acid each 5(55.6%). Whereas gentamicin and ciprofloxacin each 7(77.8%) were effective. The least bacterial isolate *Serratia* species were susceptible to all the antibiotics tested with the exception of ampicillin.

On the other hand, gram positive cocci isolated from patients, health professionals and environment were sensitive to most of the antibiotics tested. The predominant isolate coagulase negative staphylococci were resistant to ampicillin 23(20%), cotrimoxazole 27(23%), doxycycline 42 (35.9%), chloramphenicol 45(38.5%, tetracycline 57(48.7%), nalidixic acid 102(87%). ciprofloxacin 106(95.6%), erythromycin 96(82.1%), ceftriaxone 98(83.8%), and penicillin 93(79.5%) were effective for coagulase negative staphylococci. Thirty three (28%) of the coagulase negative staphylococcus isolates were resistance to methicillin.

S. aureus among the gram positive cocci demonstrated high level of resistance to nalidixic acid 58(82.9%) and tetracycline 40(57.1%). Whereas, ceftriaxone 63(90%), ciprofloxacin 60(85.7%), cotrimoxazole 56(80%), erythromycin and chloramphenicol each 55(78.6%), ampicillin 53(75.7%), and doxycycline 47(67.1%) were relatively effective against *S. aureus* isolates. A total of 70 *S. aureus* strains were isolated on samples taken from patient's surgical site infection, health professionals and various environmental samples. Of these, 17(24.0%) were methicillin resistant, while 53(76%) were methicillin sensitive (MSSA). None of the S. *aureus* strains were resistance to vancomycin.

Table 7. Antimicrobial susceptibility pattern of bacterial pathogens isolated from swabs of postoperative surgical site infections at Gondar University Hospital, November 2010 to February 2011

Bacterial isolate	Patter	Antimicrobial agents												
		GN	ERY	CIP	TE	PE	AMP	CRO	SXT	MET	NA	VN	DOX	CAF
Gram negative														
Klebsiella	S	4(16.7)	-	15(62.5)	5(20.8)	-	2(8.3)	8(33.3	4(16.7)	-	8(33.3)	-	4(16.7	4(16.7)
species	R	20(83.3)	-	9(37.5)	19(79.2)	-	22(91.7)	16(66.7)	20(83.3)	-	16(66.7)	-	20(83.3)	20(83.3)
Escherichia coli	S	8(47.1)	-	12(70.6)	2(11.8)	-	2(11.8)	14(82.4)	3(17.6)	-	5(29.4)	-	3(17.6)	8(47.1)
	R	9(52.9)	-	5(29.4)	15(88.2)	-	15(88.2)	3(17.6)	14(82.4)	-	12(70.6)	-	14(82.4)	9(52.9)
P. aeruginosa	S	4(36.3)	-	9(81.8)	2(18.2)	-	1(9.1)	5(45.6)	1(9.1)	-	1(9.1)	-	1(9.1)	1(9.1)
	R	7(63.7)	-	2(18.2)	9(81.8)	-	10(90.9)	6(54.4)	10(90.9)	-	10(90.9)	-	10(90.9)	10(90.9)
Proteus species	S	12(85.7)	-	2(14.3)	12(85.7)	-	2(14.3)	8(57.1)	8(57.1)	-	6(42.9)	-	8(57.1)	4(28.5)
	R	2(24.3)	-	12(85.7)	2(14.3)	-	12(85.7)	6(42.9)	6(42.9)	-	8(57.1)	-	6(42.9)	10(71.5)
Enterobacter	S	7(77.8)	-	7(77.8)	6(66.7)	-	2(22.2)	5(53.6)	2(22.2)	-	4(44.4)	-	4(44.4)	2(22.2)
species	R	2(22.2)	-	2(22.2)	3(33.3)	-	7(77.8)	4(44.4)	7(77.8)	-	5(55.6)	-	5(53.6)	7(77.8)
Citrobacter	S	1(25)	-	2(50)	1(25)	-	1(25	1(25	1(25	-	1(25	-	1(25	1(25
species	R	3(75)	-	2(50)	3(75)	-	3(75)	3(75)	3(75)	-	3(75)	-	3(75)	3(75)
Serratia species	S	1(100)	-	1(100)	1(100)	-	0(0)	1(100)	1(100)	-	1(100)	-	1(100)	1(100)
	R	0(0)	-	0(0)	0(0)	-	1(100)	0(0)	0(0)	-	0(0)	-	0(0)	0(0)
Gram positive														
CNS	S	92(78.6)	96(82.1)	106(95.6)	60(51.3)	93(79.5)	94(80)	98(83.8)	90(77)	84(72)	15(13)	117(100	75(64.1)	72(61.5)
	R	25(21.4)	21(17.9)	11(4.4)	57(48.7)	24(20.5)	23(20)	19(16.2)	27(23)	33(28)	102(87)	0(0)	42(35.9)	45(38.5)
S. aureus	S	58(82.8)	55(78.6)	60(85.7)	30(42.9)	58(82.8)	53(75.7)	63(90)	56(80)	53(76)	12(17.1)	69(100)	47(67.1)	55(78.6)
	R	12(17.2)	15(21.4)	10(84.3)	40(57.1)	12(17.2)	17(24.3)	7(10)	14(20)	17(24)	58(82.9)	0(0)	23(32.9)	45(21.4)
Enterococcus	S	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)
species	R	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)

GN-gentamicin, ERY- Erythromycin, CIP- ciprofloxacin, TE- tetracycline, PE-penicillin, AMP- ampicillin , CRO- ceftriaxone, MET- methicillin,   NA-Nalidixic acid, VA-Vancomycin, DOX-doxycycline, CAF- Chloramphenicol, CONS- *Coagulase negative staphylococci*, R- resistance, S- sensitive

More than half of the Gram negative isolates demonstrated evidences of multiple antibiotics resistance. For example, 22(91.7%) of *Klebsiella* species were resistant to at least five of the antibiotics tested. Eleven (64.7%) of *Escherichia coli and* 7(63.6%) of *Pseudomonas aeruginosa* were resistant to more than five of the antibiotics tested. Ten (71.4%) of *Proteus* species were resistance to at least for four of the antibiotics tested. Among the gram negative isolates 2(14.3%) *Proteus* species and 1(11.1%) *Enterobacter* species were susceptible to all of the antibiotics tested. Pan-antibiotic resistance was noted among 4(23.5%) *Escherichia coli*, 3(27.3%) *Pseudomonas aeruginosa* and 5 (20.8%) *Klebsiella* species isolates.

On the other hand more than 25% of gram positive isolates were resistant to at least five of the antibiotics. Six (8.6%), 9(12.9%) and 18(25.7) of the *S. aureus* isolates were found to be resistant for three, four and at least five of the antibiotics tested, respectively. five out of seventy isolates were susceptible to all of the antibiotics. None of the *S. aureus* isolates were pan- resistant. Coagulase negative staphylococci species have similar pattern of multiple antibiotic resistance with that of *S. aureus* isolates (Table 8).

Table 8. Multi drug resistance pattern of bacterial isolates from patients, heath professionals and hospital environmental sources, at Gondar university hospital, November 2010 - February 2011

Bacterial isolate	Total	Antibiogram pattern					
		R0	R1	R2	R3	R4	≥R5
Gram negative	**80(29.9)**	**3(3.6)**	**7(8.8)**	**4(5)**	**4(5)**	**11(13.8)**	**43(53.6)**
Klebsiella species	24(30)	0(0)	1(4.2)	0(0)	0(0)	1(4.2)	22(91.7)
Escherichia coli	17(21.3)	0(0)	2(11.8)	1(5.9)	0(0)	3(17.6)	11(64.7)
P. aeruginosa	11(13.6)	0(0)	2(18.2)	0(0)	1(9.1)	1(9.1)	7(63.6)
Proteus species	14(17.5)	2(14.3)	1(7.1)	1(7.1)	0(0)	5(35.7)	5(35.7)
Enterobacter species	9(11.3)	1(11.1)	0(0)	2(22.2)	0(0)	1(11.1)	5(55.6)
Citrobacter species	4(5)	0(0)	1(25)	0(0)	0(0)	0(0)	3(75)
Serratia species	1(1.3)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
Gram positive	**188(70.1)**	**12(6.4)**	**22(11.7)**	**50(26.6)**	**31(16.5)**	**22(11.7)**	**50(26.6)**
S. aureus	70(37.2)	5(7.1)	11(15.7	21(30)	6(8.6)	9(12.9)	18(25.7)
CN staphylococcus	117(62.2)	7(6)	11(9.4)	29(24.8	25(21.4)	13(11.1)	32(27,4)
Enterococcus species	1(0.5)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)
Total	**268(100)**	**15(5.6)**	**29(10.8)**	**54(20.1)**	**35(13.1)**	**33(12.3)**	**93(34.7)**

R0- No antibiotic resistance, R1- Resistance to one, R2-Resistance to two , R3-Resistance to three, R4-Resistance to four, ≥ R5-resistance to five and more antibiotics.

In order to locate the likely source of infection for those patients who had postoperative surgical site infection, antibiogram pattern of the isolates were used. For example, out of 59 isolates of *S. aureus* from environmental source and health professional, 13 of them had identical antibiogram pattern with isolates of patients. Hence, the environment and/or the health professionals may be the source for staphylococcal postoperative surgical site infection in this study. Identical antibiogram patterns of the isolates that can indicate the probable source of infection are shown on table 9.

Table 9. Frequency of bacterial isolates that had identical antibiogram pattern with isolates of postoperative surgical site infection, November 2010 - February 2011

Bacterial isolates of patients	Total	Source of strains that had identical antibiogram with patient isolates		
		Environment	Health professional	Total
S. aureus	11*	9	5	14
Coagulase negative staphylococci	4	2	4	6
Klebsiella species	10	3	1	4
Escherichia coli	6	2	1	3
Proteus species	9	1	0	1
Pseudomonas aeruginosa	3	0	0	0
Enterobacter species	4	2	1	3
Citrobacter species	2	1	0	1

* The sum of isolates from the different sources exceeds that of the isolates from patients, because isolates with identical antibiogram were found in more than one source.

7. DISCUSSION

In countries where resources are limited, postoperative surgical site infections remain as one of the major types of nosocomial infections (Probhakar *et al*, 1983). The successful management of patients suffering from bacterial illnesses depends upon the identification of the types of organisms that cause the diseases and the selection of an effective antibiotic against the organism in question (Brooks *et al*, 2004). Antibiotics are one of the pillars of modern medical care and play a major role in prophylaxis and treatment of infectious diseases. Cognizant of this, the issue of their availability, selection and proper use are of critically important to the global community (Brooks *et al*, 2004). This study was carried out to gain insight into the distribution and carriage rate of bacterial flora that could be of potential health risk in a hospital facility mainly of the surgical units. Thus, the data presented in this study could provide information of immediate public health importance to clinicians in Northwest Ethiopia on the selection of antimicrobial agents for prophylaxis and treatment of patients suffering from postoperative surgical site infections.

The profiles of bacterial isolates from swabs of postoperative surgical site infection in this study are consistent with previous reports in Bahir Dar (Biadglegne *et al*, 2009) and Gondar (Mulu *et al*, 2006). The organisms associated with the infections were *S. aureus* 11(22.4%), *Klebsiella* species 10(20.4 %), *Proteus* species 9(18.4%), *Escherichia coli* 6(12.2%), *Enterobacter* species and coagulase negative staphylococci each 4(8.2%), *P. aeruginosa* 3(6.1%) and *Citrobacter* species 2(4.1%). This finding is again in agreement with similar studies in Addis Ababa, Uganda and logos- Nigeria (Endalafer *et al*, 2011; Anguzu *et al*, 2007; Oguntibeju *et al,* 2004).

This result showed that *S. aureus*, *Klebsiella* species and *Proteus* species were the major bacterial pathogens associated with surgical wound infections in the study area. This result is consistent with data in Eastern Nigeria (Nwachukwu *et al*, 2009). According to CDC, *Staphylococcus aureus* is the most prevalent organism associated with surgical wound infections. Also, according to work done by Endalafre *et al*, 2011, it was reported that out of 44 surgical wound patients examined microbiologically for surgical wound infection,15.9% had *Staphylococcal* and *Klebsiella* surgical site wound infection while, 13.6% had P*roteus* infection.

Most of the patients received prophylaxis immediately before operation. The pre operative antibiotics that the patients had received were a combination of ampicillin/gentamicin/ chloramphenicol and a separate dose of ampicillin, gentamicin and others. The most probable reason for their choice being that these antibiotics had been on market for long; they are readily available and relatively cheap (WHO, 1991). However, these antibiotics were found to be less effective for most of the clinical isolates of this study.

This study was carried out to gain an insight about the distribution and profile of possible bacterial pathogens in the hospital environments. It was found that most of the inanimate objects (83.1%) in the hospital wards and operating room were variously contaminated by bacterial agents many of which are recognized pathogens. Coagulase negative staphylococci 69(68.3%) were the most frequently isolated from all the samples collected from the wards and operating room followed by *S. aureus* 31(30.7%). This finding is consistent with a study in Nigeria (Chikere *et al*, 2008). Other studies in Taiwan and Nigeria also demonstrate similar finding on patient's medical chart and x-ray machine contamination with coagulase negative staphylococci (Sing-on *et al*, 2009, Ochie *et al*, 2009). Recently, methicillin-resistant *S. epidermidis* strains and other coagulase negative staphylococci have been emerged as common nosocomial pathogens affecting immunocompromised patients carrying medical devices (Kainer *et al*, 2007). The reason of their high prevalence may be because of the fact that staphylococci are members of the body flora of health and sick individuals. These organisms can be spread by the hands, expelled from respiratory tract to immediate environment (Robert *et al*, 2011).

Gram negative organisms that comprises of 41 (28.9%) of the environmental isolate were found contaminating surfaces of some of the inanimate objects of the wards and operating rooms. Objects that were found contaminated with gram negative bacteria were sink drains, door handle, bed-frames, and floor areas. In this study it was observed that, sinks harbor more than half of the gram negative bacterial isolates consistently than do other sites (dry surface areas, e.g., walls, floors, medical equipments and tables) in patient care areas. The common bacterial isolates of sink were *Escherichia coli* 6(27.3%), *Klebsiella* species 5 (27.7%), *Pseudomonas aeruginosa* 4(18.2%), *Enterobacter*, *Citrobacter* and *proteus* species each 2(9%) and *Serratia* species 1(4.5%) were the common isolates.

Operating table, Suction machines, blood pressure apparatus, oxygen cylinder, light sources that are frequently used in the operating room during operation were found to be contaminated mainly with *S. aureus* and other coagulase negative staphylococci. This finding is in agreement with similar report in Nablus (Yahya *et al*, 1995). The habit of leaving this equipment for long periods without cleaning and proper disinfection after use is possibly responsible for this contamination. Similarly other immovable objects such as floor, wall, light switch, door handles, bed-frames and infusion stands in orthopedics, surgical ward and operating room were heavily contaminated with gram positive bacteria of the genes staphylococci. Gram negative bacteria were also identified in small proportion in some of the above mentioned objects as listed on table 5. The detection of members of enterobacteriaceae in some of the inanimate objects in the hospital environments may due to poor hygienic practice in the wards and/or visitors who attend their sick relative at the hospital may be the source of transfer of these enteric pathogens to health care facilities.

The present study also showed that the percentage of bacterial pathogen isolated from the air of surgical units of the hospital was18 (81.8 %). The isolated of bacteria were coagulase negative staphylococci 12(53.6%) followed by *S. aureus* 6(27.3%), *Pseudomonas aeruginosa* 2(9.1%), *Proteus* species and *Enterobacter* species each 1(4.5%). The pattern of the isolates in this study is consistent with a study in Sudan (Sana *et al*, 2010). Although the direct involvement of the inanimate objects in case of disease transmission is not investigated in this work, the isolation of *S. aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus* species*, Klebsiella* species and *Enterobacter* species is a concern for possible nosocomial transmission.

Some strains of S. *aureus* have been emerged as important pathogen over the last 20 year affecting primarily hospitalized patients (Boyce *et al*, 1998). In this study, *S. aureus* nasal carriage rate of health professionals and contamination of their hands with bacterial agents which may act as nosocomial pathogens were assessed. Results of the nasal swabs culture of this study indicated that health professionals carried coagulase negative staphylococcus 21(58.3)*, S. aureus,* 15(41.7%) and MRSA 3(8.3%) in their anterior nares. The finding of the present study is inline with a study in Pakistan where the prevalence of *Staphylococcus aureus*, coagulase negative staphylococci and methicillin resistant *Staphylococcus aureus* among health care

workers were 48%, 46% and 14% respectively (Kalsoom *et al*, 2008). But this is lower when compared with the carrier rate of (76%) among burns unit staff of Indian tertiary care hospital (Aravind *et al*, 2000). This difference could be due to the fact that the Indian subjects were from special unit as well as the small sample size in our case.

This study also showed that nearly 90% health professionals dominant hands had bacterial contaminations mostly with coagulase negative staphylococcus 23(56.1%), *Klebsiella* species 3(7.3%), *Escherichia coli* and *Enterobacter* species each 1(2.4%). The rate of bacterial contamination of hands of health professionals in this study is higher as compared to a study in Iran which was 39.3 % (Gholamreza *et al*, 2000). This high rate of contamination may be due to habit of infrequent hand washing and lack of proper disinfection practice of the health professionals in our hospital.

The results of antimicrobial susceptibility testing showed various percentage of resistance among the bacterial isolates from the environment, patients and health professionals. In general , more than 80% of gram negative rods were resistant to ampicillin , cotrimoxazole , doxycycline , and tetracycline. It was reported that, ciprofloxacin were effective for more than 90% of gram negative isolates in Gondar (Mulu *et al*, 2006). However, in the present study ciprofloxacin was found to be effective for more than 75% of the isolates. This sharp fall in effectiveness may be due to overuse of it as empiric treatment option for most of the patient.

Among gram negative isolates *Klebsiella* species, *Escherichia coli, Pseudomonas aeruginosa* and *proteus* species demonstrated high level of resistance to most o the antibiotics tested. Some of the strains of these gram negative bacteria were found to be pan-resistance. All of the *Klebsiella* species, 88% of *Escherichia coli*, 82% of *Pseudomonas aeruginosa* and 78.5% of *Proteus* species were multiple antibiotic resistances. Although they are not dependable for empiric treatment ciprofloxacin and ceftriaxone were relatively effective to most of the bacterial isolates. This finding was also in agreement with the findings of other studies (Biadglign *et al*, 2009; Mulu *et al*, 2006; Moges *et al*, 2002). The relative effectiveness observed in ciprofloxacin and ceftriaxone may be due to less frequent usage of them as indicated in a study in Gondar (Abula *et al*, 2004).

Majority of the *staphylococci* 153(83.4%) were multiple antibiotic resistant and these multi-drug resistance patterns had been documented already (Mulu *et al*, 2006; Kalsoom *et al*, 2008; Beyene *et al*, 2000). In the present study, gentamicin, erythromycin, penicillin, ampicillin, ciprofloxacin and ceftriaxone were found to be active against more than 75% of *S. aureus* and coagulase negative staphylococci isolates (Table 7). Overall more than 85% resistance against tetracycline and nalidixic acid was observed in *S. aureus* and coagulase negative staphylococci. Methicillin, the first semi-synthetic penicillinase-resistant antibiotic, was introduced in 1961 to target strains of penicillinase producing *S. aureus* (Woodford *et al*, 2005). In present study 24% of the *S. aureus* and 28% of coagulase negative staphylococci isolates were found to be resistant to methicillin. Similar study in Pakistan demonstrated 29% for *S. aureus* and 22% in coagulase negative staphylococcus (Brooks *et al*, 2004). In the present study, none of the staphylococci isolates were found to be resistance to vancomycin. This finding is in agreement with other study in Pakistan (Kalsoom *et al*, 2008).

Multiple antibiotics resistance was seen in 83.4% of gram positive and 87.5% of the gram negative isolates. This is high when compared to previous studies (Biadglign *et al*, 2009; Mulu *et al*, 2006; Moges *et al*, 2002). 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. According to a report by Abula on the pattern of antibiotic usage, ampicillin, tetracycline, cotrimoxazole, chloramphenicol and gentamicin were commonly used in the study area (Abula *et al*, 2004). Multiple antibiotics resistance to these commonly used antibiotics was found to be extremely high is frustrating. Most of the isolates were resistant to these antibiotics. This finding is relatively higher as compared to other studies in Gondar (Mulu *et al*, 2006; Moges *et al*, 2002) and Bahirdar (Biadglign *et al*, 2009). This may be explained by the fact that, irrational use of antibiotics for conditions that may not clinically indicate their use, over-the-counter sell of antibiotics , some new drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them hence antimicrobial resistance strains grow around.

8. Limitations of the study

Anaerobic bacteria were not investigated due to limited laboratory facilities. The study was also limited to surgical units and patients were not followed after discharge due to problems in communication and follow up. In the present study, the phenotypic method (antibiogram) of epidemiologic typing used in attempt to identify possible cross infection from health professionals and/or environmental surfaces has a drawback. Since unrelated colony of a single species can undergo evolutionary convergence to the same resistance phenotype under antibiotic selective pressure, mutation and genetic exchange.

9. CONCLUSION

These results showed that predominant causes of postoperative surgical site infections were *S. aureus*, *Klebsiella* species and *Proteus* species. Different medical equipment, environmental surfaces, air and hands of health personnel were found to be contaminated with various types of bacterial pathogens. Table surfaces, infusion stands and other movable objects used by health professionals in daily practice may be a source of nosocomial infections in this hospital. Gram positive staphylococci were more frequently isolated from the operating room and wards especially from the environmental samples and health professionals than from patients with postoperative surgical site infection. Despite the smaller sample size used for the study *S. aureus* nasal carriage among health professionals is in expected adult population range.

In this study single as well as multiple antibiotic resistance to most of the antibiotics tested were alarmingly high. This might be a reflection of inappropriate use of antibiotics, or unavailability of a guideline regarding the selection of antibiotics for prophylaxis or empiric treatment. Although not dependable ciprofloxacin and ceftriaxone can be used for empiric treatment of severe cases before culture and sensitivity test results become available.

10. RECOMMENDATION

Based on the study findings, the following recommendations are forwarded to hospital administrator, other interested governmental and nongovernmental organization and for all health professionals of the hospital.

- Postoperative wounds should not be exposed for prolonged period unduly during the course of dressing.

- If incase laboratory tests are not available, It is recommended that ceftriaxone and ciprofloxacin be used in preference to ampicillin, penicillin, tetracycline and other commonly used antibiotics in the area.

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

- Regarding *S. aureus* nasal carriage rate a comprehensive study involving all the health personnel should be conducted to represent the Gondar University Hospital population.

- Our findings demonstrate the widespread problem of antibiotic resistance among nosocomial pathogens. Continued surveillance is necessary to guide appropriate empirical therapy for postoperative surgical site infections.

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

- Samples of disinfectants and antiseptics used in wards and surgical theatres should be checked for efficiency against microbial pathogens.

- Future studies should be extended to include cultures under anaerobic conditions to establish presence of other organisms that require such environment for growth.

- In order to confirm the role of contaminated inanimate surfaces as real source of bacterial cross-infection in hospitals, further study with the aid of molecular technique and phage typing is unavoidable.

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12. ANNEXES

12.1. Data collection form

A. Environmental sample

   1. Source (object) where sample taken --.

   2. Code number---.

   3. Media used --.

   4. Organism isolated --.

   5. Drug susceptibility pattern

      5.1 Sensitive to   ---.

      5.2 Resistance to--.

      5.3 Intermediate to---.

   6. Biochemical test ---.

   7. Gram reaction result from culture --

   8. Other remarks --.

B. Sample from surgical site wound infection

   1. Name of patient ----------------------Age ----------------- Sex -------------

   2. Code number--.

   3. Type of surgery--

   4. Were you given preoperative prophylaxis

      a. Yes, what was it?   --

      b. No.

   5. Gram stains result ---.

   6. Media used ---.

   7. Organism isolated ---.

   8. Drug susceptibility pattern

8.1. Sensitive to ---

8.2. Resistant to --

8.3. Intermediate to---

9. Biochemical test --.

10. Gram reaction result ---

11. Other remarks ---.

C. **Sample from health professionals**

1. Age ----------------- sex -------------

2. Code number, Nasal swabs -----------------, Hand swabs--------------------

3. Profession ---.

4. Media used --.

5. Organism isolated ---.

6. Drug susceptibility pattern

6.1. Sensitive to --

6.2. Resistant to --

6.3. Intermediate to--

7. Biochemical test --.

8. Gram reaction result --

9. Other remarks ---.

12.2. Procedure for specimen collection and processing

A. collection and processing of specimen from surgical site infection

1. The specimen will be collected by an experienced nurse and special care will be taken to avoid contaminating the specimen with commensal organisms from the skin.

2. With sterile cotton tipped applicator stick moistened with normal saline collect sample from the infected site.

3. Label the sample as soon as possible with the patient code number

4. Inoculate in to BAP and MacConkey agar aseptically

5. Incubate the plate aerobically at 35-37 ^{o}C for 18-24 hours.

6. Examine and report the culture; look for colony characteristics and perform biochemical test.

7. Determine drug susceptibility pattern of the isolated organism

B. Collection and processing of nasal swab

1. With a sterile cotton swab moistened with sterile normal saline gently swab the inside of the nose

2. Label the sample as soon as possible with the patient code number

3. Inoculate the specimen in to BAP aseptically

4. Incubate the plate aerobically at 35-37 ^{o}C for 18-24 hours.

5. Examine and report the culture; look for colony characteristics and perform biochemical test.

6. Determine drug susceptibility pattern of the isolated organism

C. Collection and processing of environmental samples

1. Environmental samples will be taken from inanimate objects in operating room, surgical ward and orthopedics ward.

2. Using sterile cotton tipped applicator sticks moistened with normal saline collect sample from the surface of the object.

3. Role the swab over the surface of object on 1cm^2 to take sufficient sample.

4. Label the sample as soon as possible with the patient code number

5. Inoculate the specimen in to BAP aseptically

6. Incubate the plate aerobically at 35-37 ^{o}C for 18-24 hours.

7. Examine and report the culture; look for colony characteristics and perform biochemical test.

8. Determine drug susceptibility pattern of the isolated organism

D. Biochemical testing procedures

Identification of gram positive bacteria: Gram-positive cocci will be identified based on their gram reaction, catalase and coagulase tests results.

Catalase test: This test is used to differentiate *staphylococci* (+ve) from *streptococci* (-ve)

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 test and No release of bubbles-negative test

Coagulase test: This test is used to differentiate staphylococcus aureus from other staphylococcus spp.

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. interpretation

    Clumping within 10 seconds ------------------*S. aureus*

    No clumping within 10 seconds -------------other staphylococcus *species*

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

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.

2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, manitol, urea agar and motility medium.

3. Incubate at 35-37 ^{O}c 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 six biochemical tests

E. Antimicrobial sensitivity testing

Procedure

1. prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth

2. much the turbidity of suspension with turbidity standard

3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).

4. spread the inoculum evenly over the Muller-Hinton agar plate with the swab

5. using a sterile forceps or needle ,place the antimicrobial disc on the inoculated plate

6. incubate the plate aerobically at 35-37°C For 18-24 hours

7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.

8. interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard

   Sensitive – zone of radius is wider or equal to the control

   Intermediate –zone of radius is more than three mm smaller than the control

   Resistance – no zone of inhibition.

12.3. Information sheet and consent form

Title of the Research Project: Isolation and antimicrobial susceptibility testing of Bacterial Pathogens from Surgical Site Infections and Environmental Samples at University of Gondar Hospital, Northwest Ethiopia.

Name of Investigator: Aschalew Gelaw (B.Sc, M.Sc candidate)

Name of the Organization: Addis Ababa University, faculty of Medicine Department of Medical Microbiology, Immunology and Parasitology.

Introduction

You are invited to participate as study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams include one principal investigator, sample collector, two advisors one from Addis Ababa University and the other from university of Gondar. Please take as much time as you need to read or listen the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we are trying to learn more about the distribution of bacterial pathogen in hospital environment and risk of hospital acquired infection particularly postoperative surgical site infection to design effective privation and control measure.

Procedure

In order to perform the indicated study at Gondar university hospital you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. The required clinical sample will be collected by a nurse who is currently working in the surgical ward of the hospital. Then, you are requested to give your consent to the sample collector.

Potential Risks and Discomforts

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

Potential benefits to subjects and/or to the society

Based on the diagnosis result you will be treated accordingly. On the other hand, the result of the study will be beneficial to design effective prevention and control measure for nosocomial infection. Hence, you are indirectly benefiting other patients and the society in this respect.

Compensation for participation

You will not receive any payment for your participation in this research study.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. The information collected about you will be coded using numbers.

Participation and withdrawal

You can choose whether to be a part of this study or not. You may withdrawal at any time without consequences of any kind. You may also refuse to give any sample.

Person to contact

If you have any question you can contact any of the following (Investigator and Advisors) and you may ask at any time you want.

Aschalew Gelaw, B. Sc

Cell phone: +251- 09 12 877038/ 0918711787 E-mail: aschalewgelaw@yahoo.com

Patient consent form

I the undersigned health personnel / patient with Postoperative surgical site infection has been well informed about the objective of the study entitled **"Isolation and antimicrobial susceptibility testing of Bacterial Pathogens from Surgical Site Infections and Environmental Samples at University of Gondar Hospital, Northwest, Ethiopia"**. I am also told that all information obtained at any course of the study is to be kept confidential. More over I have also been well informed of my right to keep hold of, 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 and hospital access.

Therefore, with full understanding of the situations I agree to give the entire necessary information and nasal swab and hand washing (for health personnel) or wound swab (for patient) for laboratory analysis.

Name --signature--------------------.

DECLARATION

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

M.Sc candidate:

Aschalew Gelaw, B.Sc

Signature: _____ Date of submission: _____Addis Ababa, Ethiopia

Advisors:

    Solomon G/Selassie (MD, M.Sc)

    Signature: _____ Date: _____ Place: Addis Ababa Ethiopia

    Professor Moges Tiruneh (B.Sc, M.Sc, PhD)

    Signature: _____ Date: _____ Place: Gondar, Ethiopia