

Addis Ababa University
College of Health Science Department of Medical Laboratory
Sciences.



Bacterial profile and antibiotic sensitivity pattern of the isolates from operating room environments in government hospitals in Addis Ababa, Ethiopia.

By: Edosa Kebede

A thesis submitted to the Department of Medical Laboratory Sciences College of Health Science, Addis Ababa University in partial fulfillment of the requirements for masters of sciences degree in Clinical Laboratory Sciences. (Diagnostic and Public Health Microbiology Track)

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By: Edosa Kebede (B.Sc)

Advisors:

Kassu Desta: Assistant professor, B.Sc., M.Sc.

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

Gebru Mulugeta (B.Sc., M.Sc.)

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

Surafel Fentaw (B.Sc., M.Sc.)

Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

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Approved by the Examining Board

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

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LIST OF ABBREVIATIONS

CDC - Centers for Disease Control

HAI - Health care Associated Infections

HCW - Health Care Worker

ICU - Intensive Care Unit

MDR – Multi Drug Resistance

MRSA - Methicillin Resistant Staphylococcus aureus

OR – Operating Room

SSI - Surgical Site Infections

VRE - Vancomycin Resistant Enterococci

ABSTRACT

Background : Contamination of the operating theatre is a major cause of nosocomial infection. Bacterial pathogens isolated from hospital environments are also known to develop resistance to multiple antimicrobial agents.

Objective: To assess the sanitation practices of Health Care Workers (HCWs) as well as bacterial profile and the antibiotic sensitivity pattern of the isolates in operating room environments of government hospitals in Addis Ababa.

Methods: A hospital based cross sectional study was conducted from August to November 2014 in Government Hospitals in Addis Ababa. The total of 346 specimens of surfaces swabs and air samples were collected using sterile cotton tipped swabs moistened with normal saline and open plate technique. The study was also supplemented by observation and information gathered from HCWs. Data was analyzed by SPSS version 16 and the results were explained by using percentage, tables and different figures.

Result: Of the 346 samples, 47.4% (n=164) were positive for bacterial growth, 23.8% (n=39) by open plate and 76.2% (n=125) by swabbing method, respectively. A total of 221 bacterial pathogens were recovered from all samples processed in this study. Coagulase negative *Staphylococcus*, 160(72.4%) were the predominant bacterial isolates followed by *S. aureus* 33(15%). Antibiotic susceptibility pattern of bacterial pathogens showed overall sensitivity of 88.7% to ciprofloxacin and most of them had overall resistance of 42.5% to tetracycline and 33.5% to chloramphenicol. A total of 89 healthcare workers were interviewed. Majority of them 58(65.2%) had training on hand hygiene practice. Sixty four percent 57(64.0%) of the respondents routinely used an alcohol-based hand rub for hand hygiene, 59(66.3%) had ever wore at least five types of personal protective equipment (PPE) always while providing care.

Conclusion and Recommendation: In general, we found coagulase-negative *staphylococci* to be the major species contaminating the air and other surfaces in the operating rooms. This may be due to *Staphylococci* are usually human in origin and point to the restriction of traffic in operating rooms. Microbiological surveillance of operating theaters can play an important role in reducing bacterial contamination consequently preoperative infectious episodes can be reduced considerably.

1. INTRODUCTION

1.1 Background

Hospital environment play an important role in nosocomial infection, because the environment contains diverse population of microorganisms. The increased frequency of bacterial pathogens in hospital environment is associated with a background rise in various types of nosocomial infections.(1) Nosocomial infections, otherwise known as hospital-acquired infections are those infections acquired in hospital or healthcare service unit, that first appear 48 hours or more after hospital admission or within 30 days after discharge following in patient care. They are unrelated to the original illness that brings patients to the hospital and neither present nor incubating as at the time of admission. There are several reasons why nosocomial infections are even more alarming in the 21st century. These include hospitals housing, large number of people who are sick and whose immune system are often in a weakened state, increased use of outpatient treatment meaning that people who are in hospital are sicker on average, many medical procedures that bypass the body's natural protective barriers, HCWs move from patient to patient thus providing a way for pathogens to spread, inadequate sanitation protocols regarding uniforms, equipment sterilization, washing and other preventive measures that may either be unheeded by hospital personnel and the emergence of the resistant strains of microorganisms.(2)

The source and spread of organisms inside the hospital are important issues, human related organisms or the body normal flora, also found in clothing are spread through shedding during human activities .The organisms, which are particularly spread this way, include *Staphylococcus aureus*, *Micrococcus species*, alpha-hemolytic *Streptococci* and Gram-negative rods. Environmental organisms such as *Bacillus species*, *Streptomyces species* and various bacteria of non-medical importance, coming from other sources such as air dust, soil and water add to this collection. (3)

The health care environment surrounding a patient contains a diverse population of pathogenic microorganisms that arise from a patient. Surfaces in the patient care setting can also be contaminated with pathogenic organisms (e.g., from a patient colonized or infected with Methicillin Resistant *S. aureus*(MRSA), Vancomycin-Resistant *Enterococci* (VRE), or

Clostridium difficile) and can harbor viable organisms for several days. Contaminated surfaces, such as blood pressure cuffs, nursing uniforms, faucets, and computer keyboards, can serve as reservoirs of health care pathogens and vectors for cross-contamination to patients. (4)

The organisms causing most nosocomial infections may come from the patient, contaminated instrument and the environment. (5) The dissemination of health-care associated infections (HAI) often originates from cross contamination. The most common means of pathogen transference occurs between the hands of health professionals and patients. However, the hospital environment may contribute with the dissemination of pathogens. Environments occupied by colonized and/or infected patients generally can become contaminated. The presence of bacteria is common in inanimate surfaces and equipment. It was identified that in the USA there is frequent contamination of surfaces by vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA). (6)

Direct contamination is not the sole way of acquiring HAIs; contamination through environment of the patient has been identified as a possible route. Indeed, items in contact with the patient or in its vicinity may be frequently contaminated. Textiles may act as reservoirs of microorganisms, since pathogens may be able to survive on such surfaces for periods ranging from a few minutes to several months. Various species of microorganisms, including some relevant for HAIs, have been isolated from hospital textiles. (7) Common human pathogens, such as *Escherichia coli*, *Enterococcus species*, *Acinetobacter species*, and *Staphylococcus aureus* can survive for long periods on hospital surfaces or fomites that can potentially transmit infectious organisms. (8)

Contamination of operating theatres is one of the most life-threatening sources of nosocomial infection for patients. Medical staff still represents an exogenous contaminant source in operating theatres and personnel move back and forth between the operating theatre and other parts of the hospital without changing their gowns or slippers. Moreover, patients are not consistently cleaned or shaved before coming to the operating theatre. All these factors play a role in contamination of operating theatres and consequent postoperative infections. (9)

Intra-operative contamination is common in every operating room. However, despite several technological and behavioral developments, bacteria cannot be fully eliminated from an

operating room. Bacterial adhesion to and transfer between surfaces is a complicated process and with regard to the success of biomaterials implants, studies on bacterial adhesion and transfer should not be confined to biomaterials surfaces in the human body, but also encompass surfaces in the operating room, where the origin of many biomaterials related infections is found. (10)

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 (11). It is recommended that the cost of hospital infection control program should be included in the health budget of the country and fund allocated for the infection control committee for routine control purposes and to bear the cost of outbreaks. There is need for adequate staffing and continuous education of staff on the principles of infection control, especially hand washing which is the single most important effective measure to reduce the risks of cross infection.(2)

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 (12). 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 (13).

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 (14).

1.2 Statement of the problem

Nosocomial infection is a major challenge to the health care system and results in significant mortality, morbidity, and economic burden. These infections may result in substantial morbidity and higher health care costs. Intensive care unit (ICU) patients are at great risk of acquiring nosocomial infections because of breaches in host defense included as a result of trauma, invasive medical devices, and/or corticosteroid therapy. (15)

Hospital acquired surgical site infection (SSI) is one of the major health problems throughout the world and is a serious complication affecting hospitalized patients (16-19). Among hospital acquired infections, SSI accounts for 14-16% of the inpatient infections.(17) SSI is dangerous condition with a heavy burden on the patient has been associated with an increased morbidity, mortality and health care cost that have huge economic impact. (18)

A survey sponsored by World Health Organization demonstrated a prevalence of nosocomial infections varying from 3-21% with Surgical site infections accounting for 5-34%.(20)

Bacterial pathogens still play a considerable role in hospital acquired infections in Ethiopia. The prevalence of nosocomial infection was reported to be 38.5% (21) and SSI 3.5% (22) in Ethiopia.

It is documented that, the source of postoperative surgical site infections can be either endogenous or exogenous. 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. 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 (11, 23).

The emergence of resistance to antimicrobial agents is a global public health problem particularly in pathogens causing nosocomial infections. Antimicrobial resistance results in increased illness, deaths and health care costs. Treatment of these infections is frequently complicated by antimicrobial resistance, a problem that has been increasing over time. Antimicrobial resistance has become a major clinical and public health problem within the lifetime of most people living today. (4)

Despite the advance in modern medicine nosocomial infection still poses a risk of increased morbidity and mortality to patients. For this reason, 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.

The studies conducted in Addis Ababa to assess bacterial pathogens in hospital environment especially in Operation Room were very limited. Therefore, this study was undertaken to investigate bacteria profile and antibiotic sensitivity of the isolates from hospital environments with special emphasis on the surfaces in different areas of operating room in government hospitals in Addis Ababa.

In contrast to previous studies on the role of environmental colonization that were performed during nosocomial pathogen outbreaks (24), this study was conducted when there was no outbreak and reflects the regular daily risk of colonization or infection from hospital fomites. The study showed that a significant proportion of air/surfaces of Operating rooms to be heavily contaminated with bacteria. Although the relation between postoperative wound infection and cleanliness of operating room has not been investigated, our study indicated that periodic examination of the operating suite is very useful to detect contamination and the treatment of such areas may contribute to a decreased postoperative wound infection which may be caused by extrinsic factors.

2. LITERATURE REVIEW

Many studies have demonstrated the ease with which pathogenic microbes can be transferred from colonized and/or infected patients to hospital objects within the patient's room and/or from hospital objects to the hands of health care workers. There is also evidence suggesting that a patient residing in a room previously occupied by an infected or colonized individual is at a much greater risk of acquiring the microbe left by the previous occupant or from objects in the room despite adherence to established Centers for Disease Control and Prevention (CDC) infection control guidelines for terminal cleaning and hand hygiene protocols. (25)

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 potential nosocomial pathogens in a government hospital in Nigeria was conducted. Samples were obtained from doctors, nurses, 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 species* (5=8.9%). Among the Gram negative bacilli, *Escherichia coli* were the highest (4=7.1%). Others were *Klebsiella pneumonia* (3=5.3%), *Proteus species* (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 (26).

A study conducted on distribution of potential nosocomial pathogens isolated from environments of four selected hospital in Sokoto, North Western Nigeria. In this study, 160 samples were collected from different items, namely: Sink, floor, bed, bed cover, toilet floor, bed pan and ward wall. A total of 258 bacteria were isolated and identified from the hospitals. The potential pathogenic bacteria isolated in this study were *Staphylococcus aureus* 78 (30.2%) and *Proteus vulgaris* 46 (17.8%), *Pseudomonas aeruginosa* 31(12.0%), *E. coli* 30 (11.6%), *Bacillus cereus* 30 (11.6%), *Klebsiella species* 13 (5%), *Salmonella species* 11 (4.3%), *Shigella species* 10 (4%) and *Proteus mirabilis* 9 (3.5%). (1)

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 (27).

One study conducted to assess bacterial contamination of operating theatre and other specialized care unit in a tertiary hospital in Northeastern Nigeria. Of the 267 samples collected and analyzed, 70.0% (n=186) were positive for bacterial growth, 14.1% (n=26) by open plate and 85.9% (n=160) by swabbing method, respectively. Coagulase-negative *Staphylococci species* accounted for 72.1% (n=134) of bacteria pathogens isolated, followed by *Proteus species* 8.6% (n=16), *E. coli* 8.0% (n=15), *Pseudomonas aeruginosa* 6.9% (n=13) Coliforms and *Klebsiella pneumoniae* were 2.2% (n=4) each, respectively. The degree of bacterial contamination showed that coagulase-negative *Staphylococci* predominate in all units sampled, with varied frequency of occurrence of other bacteria pathogens. (28)

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 ICU 44% and surgical ward 53.3%. Other bacterial isolates were *Klebsiella species*, *Acinetobacter species*. In the study it had been concluded that the patients chair may be the source for cross infection in surgical units (29).

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 Coliforms being isolated most often (45 times; 31.7%) (30)

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, endo-tracheal 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 equipment was suggested as solution in this study (31).

A study on white coats reported 95% contamination, and indicated the presence of *Staphylococcus aureus* in 23% of the white coats; these bacteria appear to be found more specifically around sleeves and pockets. Pathogens were also found on other medical staff uniforms, or on bed linen in patients' rooms. Textiles may thus be responsible for transmission of pathogenic microorganisms to patients, as a few studies described transmission of microorganisms from uniforms to patient and bed linen, or from dirty bed linen to staff uniforms. (7)

Another study concluded that a HCWs hand became contaminated after entering a regular patients' room (one who was not on contact precautions) and only touching common surfaces close to the patient (bed rails, bedside table), without direct patient contact. The same hand contact was done by other personnel in unoccupied rooms that had been terminally cleaned after patient discharge. Ungloved hands became contaminated with low levels of pathogenic microorganisms more than 50 percent of the time, even from surfaces in rooms that had been terminally cleaned after patient discharge. (4)

A study was conducted on bacterial contamination in operating theatres of district hospital Budgam in Kashmir division. Samples were collected by open plate method and swab method for air, surface and articles, and the bacterial pathogens comprising of *Staphylococcus species*, *Bacillus species*, and Coagulase negative *Staphylococci species* were identified. (32)

Similarly, study conducted at University of Gondar Hospital, Northwest Ethiopia showed that 142 bacteria were isolated from the hospital environments such as medical devices, inanimate objects and air. Out of 142 bacterial pathogens isolated, 101 (71.1%) and 41 (28.9%) were Gram-positive and Gram negative, respectively. The most commonly isolated Gram-positive bacteria from the surgical units were coagulase negative *Staphylococci* followed by *S. aureus* and *Enterococcus species*. Similarly, *Klebsiella species*, *E. coli*, *P. aeruginosa*, *Proteus species* and *Enterobacter* were common among Gram-negative isolates. (33)

3. SIGNIFICANCE OF THE STUDY

This study was carried out to gain insight concerning bacterial profile and antibiotic sensitivity of pathogenic bacteria species that could be of potential health risk in a hospital facility.

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. This study also plays a great role in describing antimicrobial susceptibility pattern of isolates to the common antibiotics used in the area.

4. OBJECTIVES OF THE STUDY

4.1 General Objective

To assess the sanitation practices of Health Care Workers as well as bacterial profile and the antibiotic sensitivity pattern of the isolates in operating room environments of government hospitals in Addis Ababa from August to November 2014.

4.2 Specific Objectives

- To identify common bacterial pathogens contaminating the environment of operating room.
- To assess the antimicrobial susceptibility pattern of bacterial isolates.
- To determine sanitation practice of HCWs.

5. METHODOLOGY

5.1 Study Design

A hospital based cross sectional study was conducted from August to November 2014.

5.2 Study area

The study was conducted in government hospitals in Addis Ababa. Addis Ababa has a population size of 2,738,248 with annual growth rate of 2.1. The city is divided into ten sub-cities and 99 kebeles (Lowest level administrative unit in the city) (34). The city has 45 hospitals. Ten are public hospitals, of which, 5 are under Addis Ababa Regional Health Bureau (AARHB) and 5 are specialized referral ones. Three are uniformed forces (military); 4 are NGO's and the rest 28 are private hospitals. (34) The study conducted in 9 government hospitals which include St. Paul Hospital, Black Lion Hospital, Menilek II Hospital, Yekatit 12 Hospital, Zewuditu Hospital, Ras Desta Damtew Hospital, Ghandi Hospital, Tirunesh Beijing Hospital and Ethiopian Federal Police Commission Referral Hospital. The private hospitals were excluded depending on the number of operating room and the number of operation performed per day.

5.3 Source Population

All health personnel and all inanimate objects which were found in the operating room were the source population of the study.

5.4 Study Population

All staffs in operating room and all inanimate objects suspected to harbor bacterial pathogens in operating room were the study populations.

5.5 Sampling technique

The convenient sampling technique was used.

5.6 Variables of the study

Independent variables: Infection prevention practices, medical devices, various surfaces and inanimate objects such as sink, floor, operation bed, operation table, walls, telephone handle, wheelchair, equipment and air.

Dependent variables: bacterial isolates, drug susceptibility pattern (susceptible, resistance, intermediate), sanitation practices such as hand hygiene and use of PPE.

5.7 Data Collection and Processing

Data collection: Information which was relevant to the study was collected from health care workers by using questionnaire and observational check lists. Eighty nine volunteer health professionals in operating room were included and janitors were not included because they left the operating room early before the beginning of data collection. In addition, a total of 346 samples from air and inanimate objects within the Operating room that could be touched by health professionals, patients or attendants were screened for bacterial contamination in 3 rounds. The evaluation of bacterial contamination in an operating room was performed by using settle plate and swab method. The samples were collected early morning before the beginning of surgery.

(a) Settle plate method: Air sampling was performed with settle plate methods. Petri dishes containing Blood and Mac-Conkey's agar media were transported to operation theatres in

sealed plastic bags. The plates were labeled with sample number, time and date of sample collection. The plates were placed at three chosen places in the operation theatre at about 1 meter above the ground, and exposed for 15 minutes. After this exposure, the plates of air sample were covered with their lids and taken to laboratory in sealed plastic bags and incubated at 35° C for 24 to 48 hours. (32)

(b) Swab method: A swab soaked in sterile normal saline was used to collect samples from different surfaces such as the floor, walls, equipment, instruments, operation tables, sink, trolley etc. All the samples were labeled properly and transported to the Microbiology Department of Ethiopian Public Health Laboratory for processing.

Observational checklists included proper hand hygiene performance, proper PPE use, Proper environmental cleaning, reprocessing of reusable environmental devices and instruments. This observational assessment was done by trained experts.

Transportation of Samples: following collection, specimens were transported by placing each swab in a separate sterile test tube containing amies transport media to the Microbiology Department of Ethiopian Public Health Laboratory for processing. The air sample plates were covered with their lids and taken to laboratory in sealed plastic bags.

Sample processing: The swabs were inoculated into Mac-Conkey agar, blood agar and manitol salt agar. The inoculated agar plates were incubated at 35°C for 24-48 hours. Then the growth was inspected to identify the bacteria.

Presumptive identification of bacteria were based on gram reaction, colony characteristics of the organisms like hemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms.

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

Susceptibility testing: Susceptibility testing was performed on isolates based on the agar disc diffusion technique developed by Bauer et al (35). 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 (35). 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 antibiotic impregnated paper disk into the agar medium. The medium was then incubated at 35° C for 18-24 hours. The results were expressed as susceptible/intermediate/resistant according to criteria developed in 2013 by Clinical and Laboratory Standards Institute (CLSI) (36). The intermediate results were recorded as resistant. The drugs were ampicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), cefoxitin 30(µg), tetracycline 30(µg), chloramphenicol (30µg), ceftriaxone (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), methicillin (5µ g), penicillin (10IU), erythromycin (15µg), clindamycin (2µg), vancomycin (30µg) and amoxicillin-clavulanic acid (10µg).

5.8 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. Aseptic techniques were observed in all the steps of specimen collection and inoculation onto culture media to minimize contamination. All culture media were prepared according to the directions of the manufacturers. Culture media were tested for sterility and performance. Pre-analytical, analytical and post-analytical stages of quality assurance that were incorporated in standard operating procedures (SOPs) of the microbiology laboratory of Ethiopian Public Institution 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 potency of the drugs.

5.9 Ethical Considerations

Ethical clearance was obtained from the review committee of Collage of Health Science Department of Medical Laboratory Science, Addis Abba University. Ethical clearance was also taken from Addis Ababa City Health Bureau. Permission was obtained from administrators of hospitals involved.

5.10 Data Analysis

Data was analyzed by SPSS version 16 and the results were explained by percentage, tables and different figures.

6. RESULTS

6.1: General Infection Prevention Practice of HCWs.

A total of 89 healthcare workers were interviewed. From the total respondents proportions of males were 49(55.1%). Regarding their profession 69(77.5%) were nurses, 12(13.5) were anesthetists and 8(9.0%) were medical doctors. (Table- 6.1)

Respondents practices were assessed for the main element of infection prevention practice like hand hygiene, training , use of personal protective equipment, disinfection of the surfaces, availability of ventilators, cleaning of OR, and sterilization of materials .

According to the respondents majority of them 58(65.2%) had training on hand hygiene practice. 57(64.0%) of the respondents routinely used an alcohol-based hand rub for hand hygiene. Regarding to hand hygiene material more than half of respondents 51(57.3%) used soap for hand washing, whereas 21(23.6%) used hot and cold water, 6(6.7%) used antiseptic products for hand washing practice.

Majority the respondents of 59(66.3%) had ever wore at least five types of personal protective equipment (PPE) while providing patient care. All participants responded that disinfection of the surfaces performed in each operating room. Majority of respondents 80(89.9%) performed disinfection before the next operation and 5(5.6%) once a day. Concerning the disinfecting agents 57(64.0%) used bleach, 22(24.7%) used 70% alcohol and 4(4.5%) used both bleach and 70% alcohol.

All of our participants responded that there was no ventilator in Operating room of the selected hospital. According to the participants the cleaning of Operating room performed early morning before the operation began. Regarding to the type of Operating room 55(61.8%) of the participants responded both major and minor and 3(3.4%) minor.

Majority of the respondents 51(57.3%) used physical sterilization technique, 13(14.6%) used chemical sterilization technique and 25(28.1%) used both physical and chemical sterilization. Among the respondent 53(59.6 %) reported that they performed sterilization of materials before the next operation, 25(28.1%) performed once a day. (Table-6.1)

Table-6.1: Infection Prevention Practice of HCW in OR of Government Hospitals in Addis Ababa, August 2014 to November 2014.

Characteristics		Frequency	Percent
Gender	Male	49	55.1
	Female	40	44.9
Profession	Anesthetist	12	13.5
	Medical Doctor	8	9.0
	Nurse	69	77.5
Training on hand hygiene	Yes	58	65.2
	No	31	34.8
Hand rub	Yes	57	64.0
	No	32	36.0
Hand wash material	Soap	51	57.3
	Antiseptic products	6	6.7
	Hot and cold water	21	23.6
	Liquid hand wash	3	3.4
	Soap and hot and cold water	8	9.0
PPE	Use Protective Eyewear, Head cover, Foot cover, gloves and gown.	59	66.3
	Except protective eyewear	30	33.7
Disinfect the Surface	Yes	89	100.0
	No	0	0
How often disinfection performed	3 times a day	2	2.2
	Before the next operation	80	89.9
	Once a day	5	5.6
	Twice a day	2	2.2
Type of Disinfectants	70% alcohol	22	24.7
	Bleach	57	64.0
	Both	4	4.5
	70% alcohol & Others	6	6.7
Availability of ventilator	Yes	0	0
	No	89	100.0
OR cleaned	Yes	89	100.0
	No	0	0
Type of OR	Major	31	34.8
	Minor	3	3.4
	Both	55	61.8
Type of sterilization	Chemical	13	14.6
	Physical	51	57.3

	Both	25	28.1
Frequency of sterilization	Before the next operation	53	59.6
	Once a day	25	28.1
	Others	11	12.4
Total		89	100.0

All selected health care facilities and their activities related to infection prevention practices by health workers in Operating Room were observed. Infection prevention practices such as proper practice of hand hygiene, use of personal protective equipment, environmental cleaning and reprocessing of reusable instruments and devices were activities focused for observational assessment. This observational assessment was conducted in each hospital once.

Based on our assessment of hand hygiene practice of healthcare workers (HCW) in Operating Room , the highest rate of practice was observed before and after completing their procedures and PPE removal. On the other hand least practices were observed when hands moved from contaminated area to clean area during operation. Based on our observational assessment most of personal protective equipment were practiced in operation room. However, there was poor practice of removal and discarding of PPE prior to leaving the patient room. The other problem was almost all HCW wore the same gown for different patients.

We observed that environmental surfaces were cleaned. The cleaning and disinfecting materials were used according to manufacturer instruction. Instrument processing was carried out including decontamination and sterilization processes. However, in some hospitals the decontaminating solutions were not labeled recently. This means the solutions were not changed with daily base. Soiled linen was kept in linen hamper and in few areas was put in plastic pail. Clean linen was kept on the closed shelf and in the cupboard.

6. 2. Bacterial isolates of Environments.

A total of 346 specimens were collected from environments of operating room by swabbing and air by settle plate technique. Of these 266(76.9%) were from inanimate objects by swabbing and 80(23.1%) were from air by settle plate technique. One hundred sixty four (164=47.4%) of the total specimens had bacterial growth. Forty out of the 346 specimens (11.6 %) had mixed growth, while 124 (35.8 %) had pure (single) bacterial growth. The rest, 182 (52.6 %) had no bacterial growth. A total of 221 bacterial pathogens were recovered from all specimens processed during the study. Among these, 203 (91.9%) were Gram-positive and 18 (8.1%) were Gram-negative bacteria. Among the Gram positive isolates, Coagulase negative *Staphylococci* were predominant followed by *S.aureus*. *Klebsiella species* were the dominant isolate of the Gram negative rods. (Table 6.2)

Table-6.2: Profiles of bacterial isolates identified in Environments of Operating Room of Addis Ababa Government Hospitals, August 2014 to November 2014.

Bacterial Isolate	Total	Percentage
Gram Positive	203	91.9
Coagulase Negative <i>Staphylococcus</i>	160	78.8
<i>Staphylococcus aureus</i>	33	16.3
<i>Bacillus species</i>	8	3.9
<i>Micrococcus species</i>	1	0.5
<i>Entrococcus species</i>	1	0.5
Gram Negative	18	8.1
<i>Klebsiella species</i>	6	33.3
<i>Escherichia coli</i>	5	27.8
<i>Pseudomonas aeruginosa</i>	3	16.6
<i>Citrobacter species</i>	2	11.1
<i>Entrobacter species</i>	1	5.6
<i>Acinetobacter species</i>	1	5.6
Total	221	100

Table-6.3: Analysis of specimens of inanimate objects and air for the presence of bacterial pathogens at Addis Ababa Government Hospitals, August 2014 to November 2014.

Object screened	Number	Bacterial isolates		Total isolates
		Gram positive	Gram negative	
Air	80	65	0	65
Autoclave	2	0	0	0
Cesarean section sets	4	0	0	0
Chair	13	13	0	13
Door handle	23	11	0	11
Drape	4	1	0	1
Floor	25	15	1	16
Instrument table	3	1	1	2
Light source	15	4	0	4
Operation table	47	21	0	21
Oxygen Cylinder	12	8	0	8
Shelf	1	0	0	0
Sink	35	15	15	30
Small steam sterilizer	1	0	0	0
Stretcher	16	14	0	14
Telephone handle	1	4	1	5
Trolley	49	27	0	27
Wall	14	3	0	3
Wheel chair	1	1	0	1
Total	346	203	18	221

Gram negative rods especially in the family of *Enterobacteriaceae* were most frequently isolated on moisten objects such as sink (Table 6.3). From the total 18 Gram negative bacteria identified in the study, 15(83.3%) were isolated from sinks. Among these, 6(40%) *Klebsiella species*, 4(26.6%) *Escherichia coli*, 2(13.3%) *Citrobacter species* and *Pseudomonas aeruginosa*, *Entrobacter* and *Acinetobacter species* 1(6.7%) each were the common Gram negative bacteria

isolated. Whereas, gram positive bacteria in the genus staphylococci were mostly found contaminating dry surfaces. Bacterial pathogens were not identified from autoclave, small steam sterilizer, shelf and sterile materials such as cesarean section sets.

Table:6. 4 Distribution of bacterial isolates from inanimate objects and air of Operating Room of Addis Ababa Government Hospitals, August 2014 to November 2014.

Bacterial Isolate	No of inanimate object of Culture Positive(n=125) No (%)	No of Air of Culture positive(n=39) No (%)	Total (n=164)
Gram Positive	138(68.0)	65(32.0)	203(91.9%)
Coagulase Negative <i>Staphylococcus</i>	109(79)	51(78.5)	160(78.8)
<i>Staphylococcus aureus</i>	22(16)	11(16.9)	33(16.3)
<i>Bacillus species</i>	6(4.3)	2(3.1)	8(3.9)
<i>Micrococcus species</i>	1(0.7)	0(0)	1(0.5)
<i>Entrococcus species</i>	0(0)	1(1.5)	1(0.5)
Gram Negative	18(100)	0(0)	18(8.1%)
<i>Klebsiella species</i>	6(33.3)	0(0)	6(33.3)
<i>Escherichia coli</i>	5(27.8)	0(0)	5(27.8)
<i>Pseudomonas aeruginosa</i>	3(16.6)	0(0)	3(16.6)
<i>Citrobacter species</i>	2(11.1)	0(0)	2(11.1)
<i>Entrobacter species</i>	1(5.6)	0(0)	1(5.6)
<i>Acinetobacter species</i>	1(5.6)	0(0)	1(5.6)
Total	156(70.6)	65(29.4)	221(100%)

In this study 266 swab specimens were collected from various inanimate objects in the operating room. From these, 125 (47.0%) of the inanimate objects had demonstrated evidence of bacterial contamination. Twenty out of 125 (16.0%) inanimate objects had mixed bacterial growth, while 105(84.0%) had pure growth. One hundred forty one 141(53.0%) of the inanimate objects did

not show any bacteria growth. Out of one hundred fifty six bacterial pathogens isolated from inanimate objects 138 (88.5%) and 18 (11.5%) were Gram positive and gram negative, respectively. The most commonly isolated Gram positive bacteria from the swab of inanimate objects were coagulase negative *Staphylococci* 109(79%) followed by *S. aureus* 22(16%). Similarly, *Klebsiella species* 6(33.3%), *Escherichia coli* 5(27.8%), *Pseudomonas aeruginosa* 3(16.6%) were common among Gram-negative isolates. (Table 6.4)

Eighty (80) specimens were collected by settle plate from air in the operating room. From these, 39 (48.8%) had demonstrated evidence of bacterial contamination. Twenty out of 39 (51.3%) had mixed bacterial growth, while 19(48.7%) had pure growth. Forty one (51.2%) of the air did not show any bacteria growth. A total of 65 bacteria were recovered from air samples of the operating room. All of the isolated bacteria were Gram positive. The major isolate in the air sample was coagulase negative *Staphylococci* 51(78.5%) followed by *S. aureus* 11(16.9%), *Bacillus species* 2(3.1 %) and *Enterococcus species* 1(1.5 %). Fortunately, Gram negative bacteria were not isolated from air sample.

6. 3. Antimicrobial susceptibility test

The susceptibility patterns of isolates revealed varying degrees of resistance to the antibiotics tested. Gram negative rods isolated from different sample sources were highly resistant to most of the antibiotics tested(Table-6.6). The predominant isolate, *Klebsiella species* demonstrated high level of resistance to chloramphenicol and tetracycline each 5(83.3%), ceftriaxone, cefoxitin and gentamicin each 4(66.7%). Ciprofloxacin were relatively effective against 4(66.7%) of the *Klebsiella species*.

As indicated in Table-6, 60% of *E. coli* showed resistance to ampicillin, cefoxitin, tetracycline, trimethoprim- sulfamethoxazole and amoxicillin-clavulanic acid. Ciprofloxacin and ceftriaxone each 4(80%) were effective against *Escherichia coli*. *Pseudomonas aeruginosa* showed 2(66.7%) resistance to gentamicin. Ciprofloxacin was effective against *Pseudomonas aeruginosa*.

Similarly, ciprofloxacin, chloramphenicol and cefoxitin each 2(100%) were effective against *Citrobacter species*. *Acinetobacter species* were susceptible to all the antibiotics tested with the exception of gentamicin. *Enterobacter species* demonstrated high level of resistance to ampicillin, chloramphenicol, ceftriaxone and trimethoprim- sulfamethoxazole.

The major isolate coagulase negative *Staphylococci* were resistant to tetracycline 67(47.9%), chloramphenicol 52(32.5%), cefoxitin 51(31.9), penicillin 44(27.5%), trimethoprim-sulfamethoxazole 39(24.4%). Methicillin resistance was 51(31.9). Ciprofloxacin 144(90%) and clindamycin 136(85%) were effective for coagulase negative *Staphylococci*.

On the other hand, *S. aureus* were resistant to tetracycline 15(45.5%), chloramphenicol 9(27.3%) and penicillin 11(33.3%). Among the total of 33 *S. aureus*, 6(18.2%) showed methicillin resistance. Clindamycin 30(90.9%), ciprofloxacin and gentamicin each 28(84.8%) were relatively effective against *S. aureus*. *Bacillus species* were resistant to clindamycin 6(75%) and cefoxitin 5(62.5%) but susceptible to most of the drugs. (Table-6.5)

Most of the Gram negative bacterial isolates showed multi drug resistance (MDR) against at least to two or more drugs. For example, 83.3% of *Klebsiella species* and 60% of *E.coli* were resistant to at least five drugs tested. Likewise, *Citrobacter* and *Entrobacter species* were resistant to four. On the other hand, antibiogram of Gram positive isolates revealed that 25% of them were resistant to at least five drugs tested. About 16.9%, 14.4%, 26.2% of CoNS and 9.1%, 21.2%, 18.2% of *S. aureus* were resistant to three, four and at least five respectively. Despite of this, 10.6% of CoNS and 21.2% of *S. aureus* were sensitive to all antibiotics. (Table-6.7)

Table-6.5: Antibiotics susceptibility profiles of gram positive isolates from Operating Room of Government Hospitals in Addis Ababa, August 2014 to November 2014.

Bacterial isolates	Pattern	Antimicrobial agents												
		AMP	CIP	CRO	C	FOX	MET	GM	DA	E	TE	VA	P	SXT
CoNS	S	–	144	–	108	109	109	124	136	126	93	–	116	121
	%		90		67.5	68.1	68.1	77.5	85	78.8	58.1		72.5	75.6
	R	–	16	–	52	51	51	36	24	34	67	–	44	39
	%		10		32.5	31.9	31.9	22.5	15	21.2	41.9		27.5	24.4
<i>S. aureus</i>	S	–	28	–	24	27	27	28	30	26	18	–	22	26
	%		84.8		72.7	81.8	81.8	84.8	90.9	78.8	54.5		66.7	78.8
	R	–	5	–	9	6	6	5	3	7	15	–	11	7
	%		15.2		27.3	18.2	18.2	15.2	9.1	21.2	45.5		33.3	21.2
<i>Bacillus Species</i>	S	5	8	4	6	3	4	8	2	5	8	8	4	8
	%	62.5	100	50	75	37.5	50	100	25	62.5	100	100	50	100
	R	3	0	4	2	5	4	0	6	3	0	0	4	0
	%	37.5	0	50	25	62.5	50	0	75	37.5	0	0	50	0
<i>Entrococcus species</i>	S	1	1	–	–	–	–	–	–	–	1	1	0	–
	%	100	100								100	100	0	
	R	0	0	–	–	–	–	–	–	–	0	0	1	–
	%	0	0								0	0	100	
<i>Micrococcus species</i>	S	1	1	0	1	0	0	1	1	0	1	1	0	1
	%	100	100	0	100	0	0	100	100	0	100	100	0	100
	R	0	0	1	0	1	1	0	0	1	0	0	1	0
	%	0	0	100	0	100	100	0	0	100	0	0	100	0

S=sensitive, R=Resistant, %=percent, AMP=Ampicillin, CIP=Ciprofloxacin, CRO=Ceftriaxone, C=Chloramphenicol, FOX=Cefoxitin, MET=Methicillin, GM=Gentamicin, DA=Clindamycin, E=Erythromycin, TE=Tetracycline, VA=Vancomycin, P=penicillin, SXT=Trimethoprim-sulfamethoxazole.

Table-6.6: Antibiotics susceptibility profiles of gram negative isolates from Operating Room of Government Hospitals in Addis Ababa, August 2014 to November 2014.

Bacterial isolates	Pattern	Antimicrobial agents								
		AMP	CIP	CRO	C	FOX	GM	TE	SXT	AMC
<i>Klebsiella species</i>	S	–	4(66.7)	2(33.3)	1(16.7)	2(33.3)	2(33.3)	1(16.7)	3(50)	3(50)
	R	–	2(33.3)	4(66.7)	5(83.3)	4(66.7)	4(66.7)	5(83.3)	3(50)	3(50)
<i>Escherichia Coli</i>	S	2(40)	4(80)	4(80)	3(60)	2(40)	3(60)	2(40)	2(40)	2(40)
	R	3(60)	1(20)	1(20)	2(40)	3(60)	2(40)	3(60)	3(60)	3(60)
<i>P. aeruginosa</i>	S	–	3(100)	–	–	–	1(33.3)	–	–	–
	R	–	0(0)	–	–	–	2(66.7)	–	–	–
<i>Citrobacter species</i>	S	0(0)	1(50)	2(100)	2(100)	2(100)	1(50)	0(0)	0(0)	0(0)
	R	2(100)	1(50)	0(0)	0(0)	0(0)	1(50)	2(100)	2(100)	2(100)
<i>Acinetobacter species</i>	S	–	1(100)	1(100)	–	–	0(0)	1(100)	1(100)	–
	R	–	0(0)	0(0)	–	–	1(100)	0(0)	0(0)	–
<i>Entrobacter species</i>	S	0(0)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)
	R	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)

S=sensitive, R=Resistant, AMP=Ampicillin, CIP=Ciprofloxacin, CRO=Ceftriaxone, C=Chloramphenicol, FOX=Cefoxitin, GM=Gentamicin, TE=Tetracycline, SXT= Trimethoprim-sulfamethoxazole, AMC=Amoxicillin-clavulanic acid.

Table-6.7: Multi drug resistance pattern of bacterial isolates from Environments in Operating Room of Government Hospitals in Addis Ababa, August 2014 to November 2014.

Bacterial isolates	Total	Antibiogram pattern					
		R ₀	R ₁	R ₂	R ₃	R ₄	≥R ₅
Gram positive	203(91.9)	24(11.8)	32(15.8)	33(16.3)	31(15.3)	32(15.8)	51(25)
CoNS	160(78.8)	17(10.6)	26(16.3)	25(15.6)	27(16.9)	23(14.4)	42(26.2)
<i>Staphylococcus aureus</i>	33(16.3)	7(21.2)	4(12.1)	6(18.2)	3(9.1)	7(21.2)	6(18.2)
<i>Bacillus species</i>	8(3.9)	0(0)	1(12.5)	2(25)	1(12.5)	2(25)	2(25)
<i>Enterococcus species</i>	1(0.5)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)
<i>Micrococcus species</i>	1(0.5)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)
Gram Negative	18(8.1)	2(11.1)	4(22.2)	0(0)	1(5.6)	2(11.1)	9(50)
<i>Klebsiella species</i>	6(33.3)	0(0)	0(0)	0(0)	1(16.7)	0(0)	5(83.3)
<i>Escherichia coli</i>	5(27.8)	1(20)	1(20)	0(0)	0(0)	0(0)	3(60)
<i>P. aeruginosa</i>	3(16.6)	1(33.3)	2(66.7)	0(0)	0(0)	0(0)	0(0)
<i>Citrobacter species</i>	2(11.1)	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)
<i>Acinetobacter species</i>	1(5.6)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)
<i>Enterobacter species</i>	1(5.6)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
Total	221(100)	26(11.8)	36(16.3)	33(14.9)	32(14.5)	34(15.4)	60(27.1)

CoNS- Coagulase Negative Staphylococci, 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.

7. DISCUSSION

Bacterial contamination of operating theatre in hospital setting had contributed significantly to high prevalence of nosocomial infections (28). This study confirmed that various inanimate objects in the operating room theatre associated directly or indirectly with surgical procedures were variously contaminated with known bacterial pathogens. Although the direct involvement of these objects in disease transmission was not investigated in this study, the isolation of Coagulase negative *Staphylococcus* (CoNS), *S. aureus*, *Enterococcus species*, *Micrococcus species*, *Klebsiella species*, *E. coli*, *Pseudomonas aeruginosa*, *Enterobacter species* and *Acinetobacter species* presents a serious concern for possible nosocomial transmission.

This study was undertaken to investigate bacteria distribution and antibiotic sensitivity of the isolates from hospital environments with special emphasis on the surfaces in different areas of operating room. It was found that 164(47.4%) of the total samples were variously contaminated by bacterial agents many of which are recognized pathogens. Coagulase negative *Staphylococci* 160(78.8%) were the most frequently isolated from all the samples collected from the operating room followed by *S. aureus* 33(16.3%). The result of this study is similar to the report of Gelaw et al and Okon et al (33,28) which showed that coagulase negative *staphylococci* is the most frequent bacteria isolated in the operating room. Also the result of this study correlates with the report of Chikere et al. (26) which showed that *Staphylococcus epidermis* and *Staphylococcus aureus* were the most prevalent bacteria isolated in the hospital environments. It is also quite similar to the result of Ensayef et al (9) which reported that *Staphylococcus epidermis* was the most prevalent bacteria isolated in the operating theatre. This finding is also consistent with the study conducted by Nwankwo in Kano, North-western Nigeria.(37) Other studies in Taiwan and Nigeria also demonstrate similar finding on patient's medical chart and x-ray machine contamination with coagulase negative *staphylococci* (29,30). On the other hand, the result of this study is inconsistent with the work of Muhammad et al. (1) which showed that *Staphylococcus aureus* was the predominant among the isolated bacteria in hospital environments. It also contradicted the work of Genet et al (38) which reported that *Staphylococcus aureus* was the most prevalent bacteria isolated in the operating room.

The result of this study clearly spelled out, *Klebsiella species* 6(33.3%) was the most prevalent Gram negative bacteria isolated in the operating rooms followed by *Escherichia coli* 5(27.8%), *Pseudomonas aeruginosa* 3(16.6%), *Citrobacter species* 2(11.1%), *Entrobacter* and *Acinetobacter species* each 1(5.6%). This result directly contradicted the report of Muhammad et al(1) which showed that *Proteus vulgaris* was the most prevalent Gram negative bacteria isolated in the hospital environments, followed by *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella species*, *Salmonella species*, *Shigella species* and *Proteus mirabilis*. Also, it is quite different from the work of Okon et al (28) which showed that *Proteus species* was the prevalent bacteria followed by *E. coli*, *Pseudomonas aeruginosa* , and *Coliforms* and *Klebsiella pneumonia*. Alsaimary et al (39) also showed that *Pseudomonas species* were the main pathogens isolated in operating theatre. The difference in the frequency of the types of bacterial isolates between hospitals is most probably due to variation in maintaining standard personal hygiene, patients' population and sometimes departments in each hospital.(1)

Gram-negative organisms that comprises of 18(8.1%) of the total isolate were found contaminating surfaces of sink, instrument table and floor areas of the operating rooms. Our study showed that, sinks harbor about 83% of the Gram-negative bacterial isolates than other sites (dry surface areas, e.g. tables, walls and floors) in patient care areas.

Operating table, trolley, oxygen cylinder, stretcher, 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 study in Nablus. (31)

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, and door handles were heavily contaminated with Gram-positive bacteria of the genus *staphylococci*.

In this study, 39 (48.8%) positive bacterial isolates were obtained from the air sample of settle plate technique. This level of bacterial contamination simply revealed the quality of air within the sampled units. Primarily, the quality of indoor air depends on external and internal factors such as the type of ventilation system, cleaning procedures, surgical/medical team and degree of activity (40,41). The effect of external factor can be explained by the

presence of coagulase-negative staphylococci isolates that predominate in sampled units by open plate method. (28)

The bacterial profile of air sample showed that Coagulase negative *staphylococci* was the most frequently isolated species among potential pathogenic bacteria identified in OR. This finding is similar with the study conducted in Lahore(42). In this study, Gram negative bacteria were not identified from the air sample. This might be due to the inability of Gram negative bacteria to survive for a long period in the aerosolized state as it was explained by Beggs BC (43)and to resist harsh conditions like drying (44,45) as compared to their counter parts.

The results of antimicrobial susceptibility testing showed various percentage of resistance among the bacterial isolates from the environment of operating room. The inappropriate use of antibiotics, or unavailability of a guideline regarding the selection of drugs are responsible for resistance development to bacterial antimicrobials.(46)

The major gram negative isolates *Klebsiella species*, *Escherichia coli* and *Citrobacter species* demonstrated high level of resistance to most of the antibiotics tested. All of the *Klebsiella species*, *Citrobacter species* and 60% of *Escherichia coli* were demonstrated multiple antibiotic resistances. Ciprofloxacin was relatively effective to most of the bacterial isolates. This finding was also in agreement with the findings of other studies (46, 47 and 48).

On the other hand, majority of the *staphylococci* 139(72%) were multiple antibiotic resistant and these multi-drug resistance patterns had been documented already (38, 47). In this finding, clindamycin and ciprofloxacin were found to be active against more than 84% of coagulase negative *staphylococci* and *S. aureus* isolates. Quite similar with the work of Genet et al that showed 90.4% sensitivity of *S.aureus* to ciprofloxacin.(38) In this study, 18.2%) of the *S. aureus* and 31.9% of coagulase negative *staphylococci* isolates were found to be resistant to methicillin. Similar study in Northwestern Ethiopia (22) demonstrated 24% for *S. aureus* and 28% in coagulase negative *staphylococcus*.

Multiple antibiotics resistance was seen in 66.7% of Gram negative and 72.4% of the Gram positive isolates. The relatively high level of resistance to antimicrobial agents recorded in this study is a reflection of misuse or abuse of these agents in the environment. Multiple drug resistance is an extremely serious public health problem and it has been found

associated with the outbreak of major epidemic throughout the world. Thus, the multiple – drug resistance shown by these pathogens are worrisome and of public health concern. (49)

Because of the prevalent of multiple antibiotic resistant bacteria search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research.

This study also assessed important information regarding infection prevention practice of health care workers in different government hospitals in Addis Ababa. Almost all of the respondents had infection prevention practice. The study by Gulilat et al (50) showed that about 54% HCWs had infection prevention practice. This difference might be due to time gap; infection prevention training has been given for HCW now than the previous time.

In general, we found coagulase-negative *staphylococci* to be the major species contaminating the air and other surfaces in the operating rooms. *Staphylococci* are usually human in origin and point to the restriction of traffic in operating rooms. Although the relation between postoperative wound infection and cleanliness of operating room has not been investigated, this study indicated that periodic examination of the operating suite is very useful to detect contamination and the treatment of such areas may contribute to a decreased postoperative wound infection which may be caused by extrinsic factors. To achieve this aim, the proper use of disinfectant in cleaning of the various surfaces in the operating suite as well as floors is, in our opinion, a most important measure.

8.LIMITATION OF THE STUDY

The study was limited to government hospitals and their Operating rooms. Similarly, observation bias may be occurred during observational assessment.

Sample was not collected from HCWs because it is sensitive issue. For this reason, association was not done.

9. CONCLUSION

The operating theatres were contaminated with various types of bacteria. Coagulase negative *Staphylococci* were the predominant bacterial type isolated from operating rooms, followed by *S.aureus*.

The frequency of single as well as multiple drug resistance is alarmingly high. This might be a reflection of inappropriate use of antibiotics, or unavailability of a guideline regarding the selection of drugs. The presence of MDR in operating theatre may be as predisposing factor for infection. Adherence to infection prevention practices may be paramount important. Additionally, government at all tiers should endeavor to sponsor researches on development of new antibiotics that could be relevant in the treatment of severe infections caused by antibiotic resistant bacteria.

10. RECOMMENDATION

Based on the findings, the following recommendations are forwarded to hospital administrators, other stakeholders and for all health professionals of the hospitals.

- There is need for hospitals to encourage periodic review of the microbial flora of their environment and the antibiotic sensitivity pattern.
- It is also necessary that all professionals should take an active role in infection control within their organization and more resources should be provided to encourage good antibiotic Practice and good hygiene in hospitals.
- 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 is recommended.

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12. ANNEXES

12.1 Questionnaire

Questionnaire for Health Care Workers

Hello! My name is Edosa Kebede. I'm studying my second degree in Addis Ababa University. Now, I'm conducting research on bacterial profile and antibiotic sensitivity of the isolates from operating room environments in government hospitals in Addis Ababa. I need you to give me some information concerning the study.

You are indirect contact with patient on a daily basis and this is why I am interested in your opinion on health care-associated infections. Data collected will be used for research purposes only. No names shall be mentioned.

Do you agree? yes no

Hospital code: _____.

1. Gender: Male Female

2. Profession:

Nurse Medical doctor

Resident Student

Therapist Others _____

3. Did you receive formal training on hand hygiene?

yes no

4. Do you routinely use an alcohol-based hand rub for hand hygiene?

yes no

5. Hand washing materials:

- Hot and cold water Liquid hand wash Paper towels
- Soap Antiseptic products

6. Use of Personal Protective Equipment (PPE):

Protective Eyewear yes no

Head cover yes no

Foot covers yes no

Gloves yes no

Gown yes no

7. Do you use disinfectants to disinfect the surfaces?

yes no

8. If yes, how often?

Once a day

Twice a day

3 times a day

Before the next operation

Others _____

9. What type of disinfectant do you use?

70% alcohol

Bleach

Others _____

10. Is there ventilator?

yes no

11. Were the OR cleaned? Yes No

12. Type of Operation: Major Minor Both

13. Type of sterilizations used

Physical

Chemical

Others (specify) _____

14. Frequency of sterilization:

before the next operation

Once a day

Once a week

Twice a week

Others (specify) _____

Observation checklists

1. Hand hygiene performed correctly:	Practice Performed	If answer is No, document plan for remediation
a. Before contact with the patient or their immediate care environment (even if gloves are worn)	Yes No	
b. Before exiting the patient's care area after touching the patient or the patient's immediate environment (even if gloves are worn)	Yes No	
c. After contact with blood, body fluids or contaminated surfaces (even if gloves are worn)	Yes No	
d. When hands move from a contaminated-body site to a clean-body site during patient care (even if gloves are worn)	Yes No	
2. PPE is correctly used:	Practice Performed	If answer is No, document plan for remediation
a. PPE is removed and discarded prior to leaving the patient's room or care area	Yes No	
b. Hand hygiene is performed immediately after removal of PPE	Yes No	
Gloves:		
c. HCP wear gloves for potential contact with blood, body fluids, mucous membranes, non-intact skin, or contaminated equipment	Yes No	
d. HCP do not wear the same pair of gloves for the care of more than one patient	Yes No	
e. HCP do not wash gloves for the purpose of reuse	Yes No	

Gowns:		
f. HCP wear gowns to protect skin and clothing during procedures or activities where contact with blood or body fluids is anticipated	Yes No	
g. HCP do not wear the same gown for the care of more than one patient	Yes No	
Facial protection:		
h. HCP wear mouth, nose, and eye protection during procedures that are likely to generate splashes or sprays of blood or other body fluids	Yes No	
3. Environmental Cleaning	Practice Performed	If answer is No, document plan for remediation
a. Environmental surfaces, with an emphasis on surfaces in proximity to the patient and those that are frequently touched, are cleaned and then disinfected	Yes No	
b. Cleaners and disinfectants are used in accordance with manufacturer's instructions (e.g., dilution, storage, shelf-life, contact time)	Yes No	
4. Reprocessing of Reusable Instruments and Devices	Practice Performed	If answer is No, document plan for remediation
a. Reusable medical devices are cleaned, reprocessed (disinfection or sterilization) and maintained according to the manufacturer instructions.	Yes No	
b. Single-use devices are discarded after use and not used for more than one patient.	Yes No	
c. Reprocessing area has a workflow pattern such that devices clearly flow from high contamination areas to clean/sterile areas (i.e., there is clear separation between soiled and clean workspaces)	Yes No	

12.2 Data collection form

1. Source (object) where sample taken -----.
2. Code number-----.
3. Media used -----.
4. Organism isolated -----.
5. Drug susceptibility pattern
 - 5.1 Sensitive -----.
 - 5.2 Resistance -----.
 - 5.3 Intermediate -----.
6. Biochemical test -----.
7. Gram reaction result from culture -----
8. Other remarks -----

12.3 Procedure for specimen collection and processing

A. Collection and processing of environmental samples

1. Environmental samples were taken from inanimate objects in operating room, and some instruments in the hospital
2. Using sterile cotton tipped applicator sticks moistened with normal saline sample was collected from the surface of the object.
3. The swab was rolled over the surface of object on 1cm² to take sufficient sample and put in sterile test tube.
4. The sample was labeled with the object code number.
5. The samples were transported to laboratory.

6. The specimens were inoculated in to Blood agar, Mannitol salt agar and Mac-conkey agar aseptically and incubated aerobically at 35-37o C for 18-24 hours.
7. Then, the culture was examined, looked for colony characteristics, biochemical test performed and the result reported.
8. Drug susceptibility pattern of the isolated organism was done.

B. Biochemical testing procedures

Identification of gram positive bacteria: Gram-positive cocci were 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.The test organism was taken using a sterile wooden stick and added on the slide.
- 2.A drop of 3% hydrogen peroxide was added to a slide and looked for immediate bubbling.
- 3.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. A drop of physiological saline was placed on two separate slides.
2. The test organism was emulsified in each of the drop to make suspension.
3. One drop of plasma was added to one of the suspensions and mix gently. It was looked 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 were based on their test result with a series of biochemical tests.

Procedure

1. A suspension of the test organism was prepared with nutrient broth by adding 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension was inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, urea agar and motility medium.
3. Incubated at 35-37oc for 18-24 hours.
4. Looked for color change (turbidity or motility) of the medium.
5. Then, the test organism was identified by considering the results of the biochemical tests.

C. Antimicrobial sensitivity testing

Procedure

1. A suspension of the test organism was prepared by emulsifying several colony of the organism in a small volume of nutrient broth.
2. The turbidity of suspension was matched with turbidity standard.
3. With a sterile swab sample was taken from the suspension (the swab squeezed against the side of the test tube to remove the excess fluid).
4. The inoculums were spread evenly over the Muller-Hinton agar plate with the swab.
5. Using a sterile forceps, the antimicrobial disc was placed on the inoculated plate and incubated at 35-37o c for 18-24 hours.
7. The test was read after checking that the bacterial growth is neither heavy nor light. The radius of the inhibition zone was measured.
8. The reaction of the test organism to each antibiotics interpreted as sensitive, intermediate, or resistance as per the standard.

Assurance of principal Investigator.

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the principal investigator: EDOSA KEBEDE (B.Sc.)

Signature _____

Date : ____ / ____ / ____

Approval of the advisor

Name of advisor: Mr. KASSU DESTA (B.Sc., M.Sc.)

Signature _____

Date ____ / ____ / ____

Name of advisor: Mr. SURAFEL FENTAW (B.Sc., M.Sc.)

Signature _____

Date ____ / ____ / ____