CHAPTER ONE

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

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. (Aschalew&Gelaw,2011).

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 immune compromised individual.

The increased frequency of bacterial pathogen in hospital environment is associated with a background rise in various types of nosocomial infections. (Aschalew&Gelaw,2011).

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. (Aschalew&Gelaw,2011).

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. 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.

(Aschalew&Gelaw,2011).

Microbial contamination of hospital environment, especially in an operating theatre had continued to increase prevalence of nosocomial infections. With resultant effect of high morbidity and mortality rate among patient (Singh *et al*, 2013).

1.2 Objective:

General objective:

1-To isolate bacterial contaminants in operating theaters in Khartoum teaching hospital.

Specific objective:

1-To isolate and identify aerobic bacteria contaminants in operating theaters

.2-To identify the antibiotic susceptibility of the most common isolated bacterium.

1.3. Literature review

1.3.1 Definition of Nosocomial Infection:

The term “nosocomial” applies to any disease contracted by a patient while under medical care.

More precisely, nosocomial infections (NI) [also known as hospital associated/acquired infections (HAI)] are those infections that develop in a patient during his/her stay in a hospital or other type of clinical facilities which were not present at the time of admission.

It may become clinically apparent either during the hospitalization or after discharge. Hence, pathogens that cause such infections are termed nosocomial pathogens. However, an asymptomatic patient may be considered infected if pathogenic microorganisms are found in a body fluid or at a body site that is normally sterile, such as the cerebrospinal fluid or blood.

Infections acquired by staff or visitors to the hospital or other health care setting and neonatal infection that result from passage through the birth canal may also be considered nosocomial infections (Bereket *et al*,2012)

1.3.2 Bacteria

These are the most common nosocomial pathogens. A distinction may be made between:

● Commensal bacteria found in normal flora of healthy humans. These have a significant protective role by preventing colonization by pathogenic microorganisms. Some commensal bacteria may cause infection if the natural host is com- promised. For example, cutaneous coagulase- negative staphylococci cause intravascular line infection and intestinal Escherichia coli are the most common cause of urinary infection (Ducel *et al,* 2002)

● Pathogenic bacteria have greater virulence, and cause infections (sporadic or epidemic) regardless of host status.

For example: Anaerobic Gram-positive rods (e.g. Clostridium) cause gangrene. Gram positive bacteria: *Staphylococcus aureus* (cutaneous bacteria that colonize the skin and nose of both hospital staff and patients) cause a wide variety of lung, bone, heart and blood- stream infections and are frequently resistant to antibiotics; beta-hemolytic streptococci are also important. Gram-negative bacteria: Enterobacteriacae (e.g. *Escherichia coli, Proteus, Klebsiella, Enterobacter, Serratia marcescens),* may colonize sites when the host defenses are compromised (catheter insertion, bladder catheter, cannula insertion) and cause serious infections (surgical site, lung, bacteremia, peritoneum infection) (Ducel *et al*, 2002).

They may also be highly resistant. Gram negative organisms such as Pseudomonas spp. are often isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients.

Selected other bacteria are a unique risk in hospitals. For instance, Legionella species may cause pneumonia (sporadic or endemic) through inhalation of aerosols containing contaminated water (air conditioning, showers, therapeutic aerosols (Ducel *et al*, 2002).

1.3.3 Reservoirs and transmission

Bacteria that cause nosocomial infections can be acquired in several ways:

1. The permanent or transient flora of the patient

(Endogenous infection) Bacteria present in the normal flora cause infection because of transmission to sites outside the natural habitat, damage to tissueor in appropriate antibiotic therapy that allows over growth (*C. difficile,* yeast spp).For example, Gram-negative bacteria in the digestive

tract frequently cause surgical site infections after abdominal surgery or urinary tract infection in catheterized patients (Ducel *et al*, 2002).

2. Flora from another patient or member of staff

(Exogenous cross-infection) Bacteria are transmitted between patients through: direct contact between patients (hands, saliva droplets or other body fluids), in the air (droplets or dust contaminated by a patient's bacteria),via staff contaminated through patient care (hands, clothes, nose and throat) who become transient or permanent carriers, subsequently transmitting bacteria to other patients by direct contact during care, via objects contaminated by the patient, the staff's hands, and visitorsor other environmental sources (Ducel *et al*, 2002).

1.3.4. Factors influencing the development ofnosocomial infections:

1.3.4.1 The microbial agent:

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not by itself necessarily result in the development of clinical disease other factors influence the nature and frequency of nosocomial infections.

Many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may becaused by a microorganism acquired from another person in the hospital (cross-infection) or may becaused by the patient's own flora (endogenous infection).

Some organisms may be acquired from aninanimate object or substances recently contaminated from another human source. Before the introduction of basic hygienic practices and antibiotics into medical practice, most hospital infections were due to pathogens of external origin(food borne and airborne diseases, gas gangrene, tetanus,etc.) or were caused by

microorganisms not present in the normal flora of the patients (e.g. diphtheria, tuberculosis) (Ducel *et al,* 2002).

Progress in the antibiotic treatment of bacterial infections has considerably reduced mortality from many infectious diseases. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients such as *Staphylococcu saureus*, coagulase negative staphylococci, enterococci and Enterobacteriaceae (Ducel *et al,* 2002).

1.3.4.2 Patient susceptibility

Important patient factors influencing acquisition of infection include age, immune status, underlying disease, and diagnostic and therapeutic interventions (Ducel *et al,* 2002).

The extremes of life infancy and old age are associated with a decreased resistance to infection. Patients with chronic disease such as malignant tumors, leukemia, diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome (AIDS) have an increased susceptibility to infections with opportunistic pathogens (Ducel *et al,* 2002).

The latter are infections with organisms that are normally innocuous, e.g. part of the normal bacterial flora in the human, but may become pathogenic when the body's immunological defenses are compromised. Immunosuppressive drugs or irradiation may lower resistance to infection. Injuries to skin or mucous membranes bypass natural defense mechanisms. Malnutrition is also a risk (Ducel *et al,* 2002).

Many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, ventilation and suction and surgical procedures increase the risk ofinfection. Contaminated objects or

substances maybe introduced directly into tissues or normally sterile sites such as the urinary tract and the lower respiratory tract (Ducel *et al,* 2002).

1.3.4.3 Environmental factors

Health care settings are an environment where both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for patients and staff. Patients who become infected in the hospital are a further source of infection (Ducel *et al,* 2002).

Crowded conditions within the hospital, frequent transfers of patients from one unit to another, and concentration of patients highly susceptible to infection in one area (e.g. newborn infants, burn patients, and intensive care) all contribute to the development of nosocomial infections. Microbial flora may contaminate objects, devices, and materials which subsequently contact susceptible body sites of patients. In addition, new infections associated with bacteria such as water borne bacteria (atypical mycobacteria) and/orviruses and parasites continue to be identified (Ducel *et al,* 2002).

1.3.4.4 Bacterial resistance

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements, antibiotics promote the emergence of multidrug resistant strains of bacteria; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital.

The wide spread use of antimicrobials for therapy or prophylaxis is the major determinant of resistance (Ducel *et al,* 2002).

Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial agent becomes widely used, a bacterium resistant to this drug eventually emerges and may spread in the health care

setting. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective. (Ducel *et al*, 2002).

Multi resistant *Klebsiella* and *Pseudomonas aeruginosa* are prevalent in many hospitals.

This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable.

Nosocomial infections are wide spread. They are important contributors to morbidity and mortality. They will become even more important as a public health problem with increasing economic and human impact because of:

Increasing numbers and crowding of people, more frequent impaired immunity (age, illness, and treatments), new microorganisms, increasing bacterial resistance to antibiotics (Ducel *et al*, 2002).

1.3.5. Surgical wound infections (surgical site Infections)

Surgical site infection (SSI) is a major public health problem. It is the third most common health care associated infection and contributes to 13–17% of all such infections(Brigand *et a l*, 2014).

The definition is mainly clinical: purulent discharge around the wound or the insertion site of the drain, or spreading cellulitis from the wound.

The infection is usually acquired during the operation itself; either exogenously (e.g. from the air, medical equipment, surgeons and other staff), endogenously from the flora on the skin or in the operative site or, rarely, from blood used in surgery (Ducel *et al*, 2002).

SSIs are associated with considerable morbidity and it has been reported that over one-third of postoperative deaths are related, at least in part, to SSI. However, it is important to recognize that SSIs can range from a relatively

trivial wound discharge with no other complications to a life-threatening condition (UK Surgical Site Infection, 2008).

Other clinical outcomes of SSIs include poor scars that are cosmetically unacceptable, such as those that are spreading, hypertrophic or keloid, persistent pain and itching, restriction of movement, particularly when over joints, and a significant impact on emotional wellbeing (UK Surgical Site Infection, 2008).

SSI can double the length of time a patient stays in hospital and thereby increase the costs of health care (National Collaborating Center for Women's and Children's Health (UK Surgical Site Infection, 2008).

1.3.6. Sources of contamination:

The three most probable routes of infection transmission between successive sequential surgical patients are via the air, from instruments, or from environmental surfaces.

1.3.6.1 Airborne contamination:

Microbial dispersion increases with movement. Most microbes in theatre air are from staff and few from the patient. Each air change will, assuming perfect mixing, reduce airborne contamination to 37% of its former level.

A theatre should have an air change rate of around 20 air changes per hour (one air change every 3 min).Assuming 12min between the `dirty' patient leaving the theatre and the `clean' patient's wound being exposed to the theatre air, there should be under 2% of the former airborne contaminants which will then rapidly decrease further if theatre ventilation is effective, air should not be a source of infection transmission between sequential patients (Woodhead *et al*, 2002).

1.3.6.2 Surface contamination

Surfaces that do not have direct patient contact (e.g. floor, wall and light) do not become more contaminated after dirty than after clean operations.

Surfaces such as operating tables and other furniture, and instruments that make contact with more than one patient have a greater potential for transmission of infection between ‘dirty' and subsequent cases than does air.

In the absence of sterilization (autoclaving), the only practical reduction of viable microbes will be by cleaning and disinfection (Woodhead *et al*, 2002).

These decontamination processes are greatly affected by the diligence with which they are done. It seems inevitable that, when there is knowledge of an `infectious' patient, diligence will be increased.

The tradition of placing dirty cases at the end of a list facilitates this diligence (Woodhead *et al*, 2002).

If `dirty' cases (that is, patients likely to disperse microbes of particular risk to other patients) are placed last on a list, this will facilitate the process of adequate decontamination.

However, if it is judged locally that these processes can be carried out adequately during a list, there should be no extra hazard (Woodhead *et al*, 2002).

Possible (and rare) exceptions to this may be where there is profuse dispersion, for example eczema colonized with MRSA or where aerosol dispersing power tools are used on infected tissue (Woodhead *et al*, 2002).

It is recognized that in hospitals where universal precautions are practiced, it is unlikely that operating department staff will always be aware of whether a patient is likely to be `dirty', and therefore it is recommended that diligence

should be applied to cleaning the operating theatre furniture and visibly contaminated surfaces between every patient (Woodhead *et al*, 2002).

Floor:

Air born floor bacteria have been shown to account for up to 15% of CFUs; disinfection of hospital ward floors demonstrated only temporary benefit with rapid recolonization (Lidwell *et al*, 1982) large numbers of microorganism which contaminate the floors of hospital wards are commonly assumed to important sources of nosocomial infection.

Operating lamps:

The operating lamps were contaminated as the floor which indicates the contamination due to transfer of bacteria via shoes or wheel was kept at source low level and the main source was due to sedimentation of airborne bacteria carrying particles. The contamination of the wall was about half that of the floor and lamp. The light handles are attached to an unsterile light which, by its size, probably disturbs the laminar flow and creates eddies of air around it. The handle may also be inadvertently touched by the unclean heads of scrubbed personnel (Ducel *et al*, 2002).

1.3.7 Prevention and control of infection in Operation Theatre:

1.3.7.1 Ventilation

Outbreaks of Surgical Site Infections (SSIs) caused by group A beta-hemolytic streptococci have been traced to airborne transmission of the organism from colonized operating room personnel to patients , In these outbreaks, the strain causing the outbreak was recovered from the air in the operating room (Gayathri, 2008).

Operating rooms should be maintained at positive pressure with respect to corridors and adjacent areas. Positive pressure prevents airflow from less clean areas into more clean areas. Operating room air may contain

microbial-laden dust, lint, skin squames, or respiratory droplets. Air should be introduced at the ceiling and exhausted near the floor. The microbial level in operating room air is directly proportional to the number of people moving about in the room. Therefore efforts should be made to minimize personnel traffic during operations. Conventional operating room ventilation systems produce a minimum of about 15 air changes of filtered air per hour, three (20%) of which must be fresh air (Gayathri, 2008).

Laminar airflow and use of UV radiation:

Have been suggested as additional measures to reduce SSI risk for certain operations. Charnley and Eftaknan studied vertical laminar airflow systems and exhaust-ventilated clothing and found that their use decreased the SSI rate from 9% to 1%.

The findings by Lidwell *et al.*, (1987) suggest that both ultraclean air and antimicrobial prophylaxis can reduce the incidence of SSI following orthopedic implant operations, but antimicrobial prophylaxis is more beneficial than ultraclean air.

Laminar airflow can be directed vertically or horizontally, and recirculated air is usually passed through a high efficiency particulate air (HEPA) filter HEPA filters remove particles ≥0.3mm in diameter with an efficiency of 99.97%. Intra operative UV radiation has not been shown to decrease overall SSI risk used in hospitals for decontaminating air .It sterilizes the cabinet or room at low concentration, 1 per cent (Gayathri, 2008).

1.3.7.2 Environmental surfaces:

Environmental surfaces in operating rooms (e.g., tables, floors, walls, ceilings, lights) are rarely implicated as the sources of pathogens important in the development of SSIs. Nevertheless, it is important to perform routine cleaning of these surfaces to reestablish a clean environment after each

operation. There are no data to support routine disinfecting of environmental surfaces or equipment between operations in the absence of contamination or visible soiling. When visible soiling of surfaces or equipment occurs during an operation, an Environmental Protection Agency (EPA) approved hospital disinfectant should be used to decontaminate the affected areas before the next operation. This is in keeping with the Occupational Safety and Health Administration (OSHA) requirement that all equipment and environmental surfaces be cleaned and decontaminated after contact with blood or other potentially infectious materials (Gayathri, 2008).

Wet vacuuming of the floor with an EPA-approved hospital disinfectant is performed routinely after the last operation of the day or night. Care should be taken to ensure that medical equipment left in the operating room be covered so that solutions used during cleaning and disinfecting do not contact sterile devices or equipment (Gayathri, 2008).

1.3.7.3 Disinfection of patient equipment:

Disinfection removes microorganisms without complete sterilization to prevent transmission of organisms between patients. Disinfection procedures must:

Meet criteria for killing of organisms, have a detergent effect, act independently of the number of bacteria present, the degree of hardness of the water, or the presence of soap and proteins (that inhibit some disinfectants) (Ducel *et al*, 2002).

To be acceptable in the hospital environment, they must also be:

Easy to use, non-volatile, not harmful to equipment, staff or patients, free from unpleasant smells, effective within a relatively short time (Ducel *et al*, 2002).

Different products or processes achieve different levels of disinfection.

These are classified as high-, intermediate- or low-level disinfection.

High-level disinfection (critical)

This will destroy all microorganisms, with the exception of heavy contamination by bacterial spores (Ducel *et al*, 2002).

Intermediate disinfection (semi-critical)

This inactivates *Mycobacterium tuberculosis*, vegetative bacteria, most viruses and most fungi, but does not necessarily kill bacterial spores (Ducel *et al*, 2002).

Low-level disinfection (non-critical)

This can kill most bacteria, some viruses and some fungi, but cannot be relied on for killing more resistant bacteria such as *M. tuberculosis* or bacterial spores (Ducel *et al*, 2002).

These levels of disinfection are attained by using the appropriate chemical product in the manner appropriate for the desired level of disinfection (Ducel *et al*, 2002).

Sterilization:

Sterilization is the destruction of all microorganisms and can be achieved by either physical or chemical means (parker, 1978).

1.3.7.4. Conventional sterilization of surgical instruments Inadequate sterilization of surgical instruments has resulted in SSI outbreaks .Surgical instruments can be sterilized by steam under pressure, dry heat, ethylene oxide, or other approved methods. The importance of routinely monitoring

the quality of sterilization procedures has been established. Microbial monitoring of steam autoclave performance is necessary and can be accomplished by use of a biological indicator. Detailed recommendations for sterilization of surgical instruments have been published (Gayathri, 2008).

1.3.7.5 Flash sterilization of surgical instruments

The Association for the Advancement of Medical Instrumentation (1996) defines flash sterilization as "the process designated for the steam sterilization of patient care items for immediate use." During any operation, the need for emergency sterilization of equipment may arise (e.g., to reprocess an inadvertently dropped instrument). However, flash sterilization is not intended to be used for either reasons of convenience or as an alternative to purchasing additional instrument sets or to save time. Also, flash sterilization is not recommended for implantable devices because of the potential for serious infections (Gayathri, 2008).

1.3.7.6 Antimicrobial prophylaxis

Antibiotic prophylaxis is used only when it has been documented to have benefits which outweigh risks.

Some accepted indications include: selected surgical prophylaxis and Endocarditis prophylaxis (Ducel *et al*, 2002).

Where chemoprophylaxis is appropriate, antibiotics must be initiated intravenously within one hour prior to the intervention. It is often most efficient to order therapy given at call to the operating room or at the time of induction of anesthesia. In most cases, prophylaxis with a single preoperative dose is sufficient (Ducel *et al*, 2002).

The regimen selected depends on the prevailing pathogen(s), the pattern of resistance in the surgical service, the type of surgery, the serum half-life of the antibiotic, and the cost of the drugs.

Administration of prophylactic antibiotics for a longer period prior to the operation is counterproductive, as there will be a risk of infection by a resistant pathogen.

Antibiotic prophylaxis is not a substitute for appropriate aseptic surgical practice (Ducel *et al*, 2002).

CHPTER TWO
2. MATERIALS AND METHODS

2.1 Type of study:

Analytical and cross-sectional study.

2.2 Study area:

This study was conducted in Khartoum in laboratory of microbiology of Sudan University of science and technology.

2.3 Sample size:

Fifty swabs samples were collected from certain sites in operating theaters, beds, trolleis, floors, air, and focusing lamps.

2.4 Materials:

2.4.1 Media:

1. Blood agar
2. MacConkey agar
3. KIA medium
4. Urea agar
5. Peptone water
6. Simmon's citrate
7. DNase medium
8. MSA medium
9. Muller Hinton agar

2.4.2 Glass wares:

1. Petri dishes
2. Tubes
3. Slides

2.4.2 Others:

1. Benzen burner
2. Straight loop, wire loops and forceps
3. Cotton wool swabs
4. Antibiotics discs

2.5 Collection of samples:

Cotton-tipped swabs moistened with sterile peptone water were used to swab the sites in the operating theaters.

2.6 Isolation:

The swabs were directly inoculated on blood agar and MacConkey's agar near benzene burner. The preparation of media are in appendix (1and2).the pairs of inoculated media were incubated aerobically at 35-37C for 24 hours and then examined for bacterial growth.

2.7 Identification:

The colonies which obtained were purified by sub culturing in MacConkey's and blood agar. The both inoculated media were incubated aerobically at 37C for 24 hours and then examined.

2.7.1 Gram's stain:

Smear was done from over night isolate by sterile loop take small portion from colony to drop of normal saline on clean dry slide then mix and spread in circular manner. Then slide was left to dry and fixation was done by gentle heat. Crystal violate was added to smear for 1minute,and then washed by tap water ,logul's iodine was added for 1minute.then washed by tap water ,aceton alcohol added for seconds and washed by tap water .finally ,the smear covered by saffranin for 2 minutes , and washed by tap water, the smear was left to dry by air ,a drop of oil was added and examined under

light microscope(carl zeiss) by oil lens x100 (Cheesbrough, 2006).The components seen in appendix(3).

2.7.2 Biochemical tests:

Group of tests were done to identify bacteria which include the following tests:

2.7.2.1 Kligler's iron agar:

By using sterile straight loop the organism under test was inoculated onto the bottom of KIA tube and zigzag on the slope was made. The tubes were incubated over night at 37℃ (Cheesbrough, 2006).The components and preparation in appendix (4).

2.7.2.2 Indole test:

By using of sterile wire loop the organism under test was inoculated in peptone water. The tubes were incubated over night at 37° C .A drop of kovac's reagent was added after incubation period (Cheesbrough, 2006).The components and preparation are in appendix (5).

2.7.2.3 Citrate utilization test:

By using sterile straight loop the organism under test was inoculated in Simmon's citrate. The tubes incubated over night at 37° c (cheesbrough,2006). The components and preparation are in appendix (6).

2.7.2.4 Urease test:

By using sterile straight loop the organism under test was inoculated in urea ager. The tubes incubated over night at 37° c (Cheesbrough, 2006). The components and preparation are in appendix (7).

2.7.2.5 Oxidase test:

By using wooden stick small portion of the colony of tested organism was placed in oxidase disk, presence of purple color was indicated of oxidase positive and no change in color was indicated of oxidase negative.

2.7.2.6 Catalase test:

By using wooden stick several colonies of tested organism were immersed in tube with 2-3 ml of 3%H2O2, presence of air bubbles was indicated of catalase positive and no presence of air bubbles was indicated of catalase negative(Cheesbrough, 2000).The components and preparation are in appendix (10).

2.7.2.7 Deoxyribonuclease (DNase) test:

The tested organism was cultured on a medium which contains DNA making heavy spots , then incubated over night at 37˚c and colonies were tested by flooding the plate with a weak hydrochloric acid (HCL)which precipitate unhydrolyzed DNA. Presence of clear zone surrounded the colonies was indicated positive result .negative result showed no clear zone surrounded the colonies (Cheesbrough, 2000). The components and preparation are in appendix (8).

2.7.2.8 Manitol salt agar (MSA):

By using sterile wire loop under aseptic condition the organism under test was inoculated on mannitol salt agar plates making streaking. The plates were incubated at 37˚c for overnight. The positive result indicated by change in medium color to yellow and negative result indicated by no change in medium color (Cheesbrough, 2000).The components and preparation are in appendix (9).

2.7.2.9 Susceptibility testing:

Susceptibility testing was performed on isolates based on the agar disc diffusion technique. The suspension of the test organism were prepared by picking parts of similar test organisms with a sterile wire loop and suspended in sterile broth .

The densities of suspension to be inoculated were determined by comparison with opacity standard on McFarland 0.5 Barium sulfate solution. 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 and exposed to a concentration gradient of antibiotic diffusing from antibiotic impregnated paper disk into the agar medium. The medium was then incubated at 35oC 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(Aschalew&Gelaw , 2011).

Antimicrobial disc:

Commercial disc 6mm in diameters were used.

The following antibiotics were used:

Ciprofloxacin, Gentamicin, Cotrimixazole, Amikacin and Chloramphenicol.

Statistical analysis:

Data were analyzed by using statistical package for social sciences. It was analyzed by descriptive statistic for frequency.

CHAPTER THREE
3. Results

Morphological and Biochemical characteristics of isolated bacteria:

The results in table 4.1illustrate the different species of bacteria isolated from the operating theaters of KTH. While 63.6% of the isolated bacteria were Gram-negative bacilli, 36.4% were Gram-positive cocci and bacilli.

Table 4.2 summarizes the biochemical characteristics of Gram- negative bacteria, whereas table 4.3 summarizes the biochemical characteristics of Gram-positive bacteria.

Identification of isolated bacteria:

The incidence of positive cultures was 22/50 cultures(44%),table 4.4 explains identification of isolated bacteria from operating theaters, which revealed that *pseudomonas aeruginosa* was the most common isolate (63.6%),followed by *micrococcus.spp* (22.7%),*bacillus.spp*(9.2%),and *staphylococcus aureus*(4.5%).

Table 3.1 Bacteria morphology and Gram staining:

Staining	Number	Percentage
Gram positive	8	36.4%
Gram negative	14	63.6%
Total	22	100%

Table 3.2 Biochemical characteristic of isolated gram positive bacteria:

Suspected organism	catalase	Coagulase	DNase	Manitol fermentation
Staphylococcus aureus	+ve	+ve	+ve	+ve
Micrococcus spp	+v	Not done	Not done	Not done
Bacillus spp	+ve	Not done	Not done	Not done

Table 3.3 Biochemical characteristic of isolated gram negative bacilli:

Organism	Indole	Urea	Citrate	Oxidase	KIA			
P.aeruginosa	-	-	+	+	But	Slope	Gas	H2s
					R	R	-	-

+ =positive - =negative

Y =yellow R=red

Table 3.4 Number and percentage of bacterial species isolated from operating theaters:

Bacteria	Number of isolate	Percentage
Ps.aeruginosa	14	63.6%
micrococcus.spp	5	22.7%
bacillus .spp	2	9.2%
Staph. aureus	1	4.5
Total	22	100%

Table 3.5 Percentage of bacterial contamination on screened objects in operating theaters:

Objects screened	Frequency	Percentage
Bed	8	36.4%
Trollies	7	31.8%
Floor	4	18.2%
Operating lamps	2	9.1%
Air	1	4.5%

Table 3.6 Percentage of contamination rate in each operating theaters:

Operating theater	No of isolate	Percentage
Gynecological surgery	15	68.2%
Pediatric surgery	7	31.8%

Antimicrobial susceptibility test:

The result of antimicrobial susceptibility pattern of the predominant isolated *P.aeruginosa* is shown on table 3.7 below.

42.8%isolates of *Pseudomonas aeruginosa* demonstrated high level of resistance to Chloramphenicol, 21.4 % of them were resistant to cotrimixazole and amikacin, 28.6 % resistant to co-trimixazole only and 7.1% resistant to amikacin only. While 100% of *Pseudomonas aeruginosa* isolated were sensitive to ciprofloxacin and gentamicin.

Table 3.7 Antimicrobial susceptibility pattern of *P.aeruginosa*:

Bacterial isolates	**pattern**	**GN**	**CIP**	**COT**	**C**	**AK**
P.aeruginosa	S	15	21	16	18	17
	R	12	15	10	12	14

GN:gentamicin CIP:ciprofloxacin COT:cotrimixazol

C: Chloramphenicol AK: amikacin.

S:sensitive R:resistant.

Table 3.8 percentage of sensitivity and resistance to each antibiotic:

Antibiotic	**Sensitive**		**Resistant**		**Total**
	No	**%**	**No**	**%**	**14**
Gentamicin	14	100%	0	0%	14
Ciprofloxacin	14	100%	0	0%	14
Cotrimixazole	7	50%	7	50%	14
Chloramphenicol	8	57.1%	6	42.9%	14
Amikacin	13	92.9%	1	7.1%	14

CHAPTER FOUR
4. DISSCUSION

Assumingly, the operating theaters in any hospital should be situated in a strictly sterile area therefore, and obviously, bacterial contamination of these surgical theaters highly dangerous to the patients and performed huge hazard to the health care authorities.

The associated structures of the operation theaters are uniquely predisposed to contamination by various microorganisms mainly bacteria, viruses, fungi and parasites.

The results were in disagreement with these obtained by Ensayef *et al*.,(2009) who reported that common bacterial contaminants in operating theaters in Al Imam Ali Hospital in Baghdad, during 2001 was *S.epidermidis* 39.1%,*p.areuginosa* 30.4% and coliform 13.0% and rat of positive cultures was 3.7%.The recent study of Ensayef *et al*.,(2009) in Baghdad reported common bacterial contaminants in operating theaters during 2002 were coliform as Gram-negative bacteria and *S.epidermidis* as predominant Gram-positive bacteria, The prevalence of S.epidermidis was 8.3% and coliform was 62.5% and the rate of positive culture was 4%.

The results were not in accordance with those reported by Sepherhri *et al*,.(2009)in Iran who reported the most predominant contamination was *S.epidermidis*,that represented about 77% of bacterial contamination ,followed by *S.aureus* 12.5% and *Klebsiella* 2.1%.

The results were in disagreement with those obtained by BioInfo Bank institute (2010) who reported the common bacterial contaminants in operating theaters in Alwehda Educational Hospital, in Thamar ,Yemen during 2009 was *S.aureus* as Gram-positive bacteria and coliform as Gram-negative. The prevalence of *S.aureus* was 66.6% and coliform was 14.3%

and the rest was fungi; the rate of positive culture was 19.1%.

In this study, were able to isolate *bacillus spp* and *micrococcus spp*, which was not reported by any of the previous studies. The variations in the results were unknown, but could be due to sample size, used by the previous investigators (Ensayef *et al*, 2009).furthermore, personal hygiene, the safety, cleaning methods, social level of patients, operating room ventilation, sterilization methods, surgical technique, and availability of antimicrobial prophylaxis could make the observed difference.

The highest percentage of contamination was caused by *P.aeruginosa* 63.6%.the possible source of contamination of *P.aeruginosa* was antiseptic solutions or from breast-fed babies, especially this bacterium is an opportunistic pathogen that can be found in most moist environments, in addition, it has a combination of properties such as its ability to survive and spread in hospital environments, acquisition of multiple virulence determinants and intrinsic resistance to commonly used antibiotics and disinfectants. This makes *P.aeruginosa* a major nosocomial pathogen that is responsible for many outbreaks in operating theaters (Bellido and Hancock, 1993).

Followed by *micrococcus spp* is human related organisms or the body normal flora, also found in clothing are spread mechanical movement within the enclosed space.

In through shedding during human activities (Ekhaise *et a*l 2008) Identification as *Micrococcus*.spp was based on typical Gram stain morphology with large gram-positive cocci in tetrads.

Bacillus spp a vast group of hardy spore forming species that live in soil and are found in the environment Bacillus produces an emetic exotoxin capable of inducing disease in man (Silman *et al*, 1987).

The contamination by *S.aureus* was usually exogenous from surgical staff (30% *S.aureus* as nasal carrier) and from air.

The rate of contamination in operating theaters was high in the beds (36.4%). followed by Trollies (31.8%), floor (18.2%), operating lamps (9.1%), and air (4.5%). This could be explained by the fact that all of these sources have direct contact with surgical staff or patients, with potential endogenous and/or exogenous bacterial contaminant.

The antibiotic sensitivity of *P.aeruginosa* was show highly resistant to some antibiotics. This could be due to the wide spread antibiotics that routinely used in hospitals. Furthermore, strains in hospitals are often resistant to multiple antibiotics. This resistant de to acquisitions of plasmids carrying several genes that encode the enzymes that mediate resistance. (Levinson,2004).

CHAPTER FIVE
5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Different species of bacteria were isolated from operating theaters; the contamination dispends on the materials and devices used for cleaning and disinfection.

P.aeruginosa, bacillus spp, micrococcus spp and *S.aureus* were found to be the most contaminating bacteria in operating theaters and this act as a dangerous source of nosocomial infection and life threating to patients and hospital staff. This might indicate that the sterilization methods are not efficient enough, which can put the patients at risk of post-operative infection.

5.2 RECOMMENDATIONS

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

2-Continuous monitoring programs using advanced techniques (e.g. RT-PCR) for isolation and identification of bacterial contaminants in operating theaters are highly important to solve this problem.

3-Implementation of comprehensive infection control programs and surveillance of infection, in hospitals by infection control committee. Health education of hospital staff, in order to protect themselves and the patients from the contaminating bacteria, as well as from spreading pathogenic bacteria themselves.

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

5-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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Appendices

Appendix {1}: blood agar:

Content:

Nutrient agar	95ml
Sterile defibrinated blood	5ml

Weight the nutrient agar is used, at a concentration of 2.8gm in a 95 ml distilled water.

Preparation: prepared 95ml from nutrient agar is above, cooled to 50 c, added aseptically 5ml from the sterile blood and mixed gently poured in sterile Petri dishes.

Appendix[2] macConkey agar [himedia labrotaries pvt.ltp. Mumbai India]

Ingredients:	gms/litter
Peptic digest of animal tissue	17.00
Protease peptone	3.00
lactose	10.00
bile salts	1.50
sodium chlorine	5.00
neutral red	0.03
agar	15.00

preparation:

suspend 51.53 grams in 1000 ml distilled water. heat is to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 ibs pressure [121c] for minute. Mix well before pouring.

Appendix [3]: gram's stain

1.crystal violate	20 gm
2.ammonium	99 gm

3ethanol	95 gm
D.W	1L

2-Lugols iodine:

Potassium iodine	20 gm
Iodine	10 gm
Distilled water	1L

3-Aceton –alcohol decolrizer:

Acetone	500 ml
Ethanol or methanol absolute	465 ml
Distilld water	25 ml

4-Safranin :

Safranin	0.54 g
Distilled water	100 ml

Appendix [4]: kligler iron agar (KIA)(himedia laboratories pvt.ltd. Mumbai India).

Ingredients:	gm/liters
Peptic digest of animal tissue	15.00
Beef extract	3.00
Proteose peptone	3.00
Lactose	5.00
Dextrose	10.00
Ferrous sulphate	1.00
Sodium chloride	0.20
Sodium thiosulphate	5.00
Phenol red	0.30
Agar	0.30

-final PH (at 25c)7.4

Preparation :

Suspend 42.524 grams in 1000 ml distilled water. Sterilize by autoclaving at 15ibs pressure (121c) for 15 mins .Cool to about 50c .mix well and pour into sterile tubes. Allow the medium to solidify in a slope position to give a butt and slope.

Appendix [5]: peptone water(himedia laboratories pvt.ltd. Mumbai India).

Ingredients:	gm/liters
Peptic digest of animal tissue	10.00
Sodium chloride	5.00

Final PH (at 25c)7.2± 0.2

Preparation :

Suspend 15.0 grams in 1000 ml distilled water.mix well and dispense into tubes. Sterilize by autoclaving at 15 ibs pressure (121c)for 15 mins.

Appendix [6]:Simmon's citrate:

Ingredients:	gm/liters
Maganisium sulphate	0.20
Ammonium hydrogen phosphate	1.00
Di potassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00

Final PH (at 25c)6.8

Preparation:

Suspend 24.28 grams in 1000 ml distilled water. heat it to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 ibs pressure (121c)for 15 mins.

Appendix [7]:

urea agar base (Christensen))(Himedia laboratories pvt.ltd. Mumbai india).

Ingredients :	gm/litrs
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00
Di sodium phosphate	1.20
Mono potassium sulphate	0.80
Phenol red	0.012
Agar	15.00

-final PH (at 25c)6.8±0.2

Preparation:

Suspend 240.0 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 ibs pressure (115c)for 20 mins. Cool to 15 c and aseptically 15 ml of sterile 40% urea. Mix well and pour into sterile tubes.

Appendix [8]: DNA medium:

Content: casein in enzyme hydrolysate 15g papaic digest of soya benmed 5.00 g and ager 15.00 g

-final ph (at 25c) 7.3±0.2.

Weight: the medium is used at a conc. of 2.8 in 100ml distilled water.

Preparation:

suspend 2.8g in 100ml distilled water sterilized by autoclave at 15ibs pressure 121□c for 15 minutes, cooled to 50 55□c mixed well and poured in sterile petri dishes.

Appendix [9]: Mannitol salt agar:

Content:

Proteosepepetone 10,00g	
Beef extract	1.00g
Phenol red	0.025g
Sodium chloride	75.00g
D.manitol	10.00g
Agar	15.00g
Final PH at [25□c]	74±0.2

Weight the medium is used at a concentration of 11.1g in 100ml distilled water.

Preparation:

Suspend 11.1g in 100ml distilled water sterilized by autoclave at ibs pressure 121□c for 15 minutes, cooled to 50◦c mixed well and poured in sterile petridishes.

Appendix [10]: Reagents:

1.kovacsreagent :

[p] di methly1 aminobenzaldehyde 2gm

2. Hydrogen peroxide [3% H_2O_2]:

3ml H_2O_2 IN 100ml D.W

3. Hydrochloric acid:

Concentrated HCL 8.6ml and 100ml D.W.

Appendix[11]: physiological saline [0.85%]:

Nacl	0.85gm
Distilled water	100ml

Appendix[12] : the module:

- Incubator [GALL ENK AMP GE, U.K.] .
- Autoclave [Graffin and Italy George Ltd.].
- Hot air oven [leader Engeneering, U.K.].

Appendix [13]:Muller Hinton agar:

Beef infusion form	300g
Casein acid hydrolysate	17.5g
Starch	1.5g
Agar	17g
Distilled water	100ml

Disc diffusion method on Muller Hinton agar and the zone of inhibition was measured by millimeter ruler.

Biochemical characteristic of *Pseudomonas aeruginosa*