Sudan University of Science and Technology

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Isolation of Bacterial Contaminants from Operating Theatres in Khartoum

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A dissertation submitted in partial fulfillment of the requirements of M.Sc degree in Medical Laboratory Science (Microbiology)

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الآيـــــة

بسم الله الرحمن الرحيم

قال تعالى:

﴿اللهُ لاَ إِلَهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ﴾

صدق الله العظيم

سورةالبقرة - الآية (255)

Dedication

To my Parents

To my brothers and sisters

To my friends

To everyone who supported me

Acknowledgments

First of all I thank Almighty Allah for giving me the strength to complete this work. With great deal of respect I would like to thank Dr. Abdelbagi Elnagi Mohammed, my supervisor for his patience, guidance, support and valuable advice through the preparation of this study. My thanks and appreciations to Khartoum North Teaching Hospital and Khartoum Teaching Hospital laboratory staff for helping me collecting the samples for this study.

Finally I would like to thank my colleagues and my friends Reham and Islam for their support and encouragement to complete this thesis.

Abstract

The main aim of this study was to isolate and identify the most common bacterial contaminants in operating theatres in Khartoum State Hospitals during the period from January to April 2010. Briefly, one hundred samples (n=100) were collected from different sites of the operating theatres. The samples were collected from the major complex theatres at Khartoum North Teaching Hospital (KNTH) and Khartoum Teaching Hospital (KTH). By employing standard morphological and biochemical techniques, both Gram-positive and Gram-negative bacteria were isolated and identified.

The common bacterial contaminants isolated from operation theatres were *Staphylococcus aureus* (30.8 %) , *Staphylococcus epidermidis* (20.5%), *Staphylococcus saprophyticus* (5.1%), *Escherichia coli* (20.5%), *Pseudomonsa aeruginosa* (15.4%), *Proteus mirabilis* (5.1%) and *Enterobacter cloacae*(2.6%). The highest contaminants were isolated from the beds (17.9%), followed by floors (15.4%), trollis (12.8%), shoes and air conditions (10.3%), suckers, focusing lamps and anaesthesia machines (7.7%), oxygen cylinders and air (5.1%).

While all *S. aureus* isolated from KNTH (100%) were methicillin resistant, only (50%) were methicillin resistant in KTH. However, 50% of *S. aureus* isolated from KTH and KNTH were sensitive to erythromycin, gentamicin and co-amoxiclav. *S. saprophyticus* was resistant to ampicillin but sensitive to erythromycin, gentamicin and co-amoxiclav. Similarly, *S. epidermidis* was sensitive to gentamicin and co-amoxiclav; however *E. cloacae* was resistant to ampicillin, co-amoxiclav and sensitive to chloramphenicol. In KNTH *P. mirabilis* was sensitive to ampicillin, co-amoxiclav and resistant to chloramphenicol and gentamicin. In contrast, *P. mirabilis* isolated from KTH was resistant to ampicillin, co-amoxiclav and gentamicin, but sensitive to chloramphenicol.

E. coli isolated from KTH was highly resistant to ampicillin and co-amoxiclav, and sensitive to gentamicin and chloramphenicol, while that isolated from KNTH, 83.3% were resistant to ampicillin, chloramphenicol and gentamicin, and 66.7% were sensitive to co-amoxiclav. While 100% of *P. aeruginosa* isolated from KNTH were resistant to chloramphenicol, 66.7% of them were resistant to ampicillin, gentamicin and co-amoxiclav. However, 100% of *P. aeruginosa* isolated from KTH were resistant to ampicillin and 66.7% of them were resistant to co-amoxiclav, but sensitive to gentamicin and chloramphenicol.

In conclusion, isolation of different resistant bacterial isolates from different surgical sites in various hospitals is a living evidence that various serotypes of bacterial species are persistant contaminants of the operating theatres in Khartoum hospitals.

ملخص البحث

الهدف الأساسي من هذة الدراسة هو عزل و تحديد الملوثات البكتيرية شيوعاً في غرف العمليات في مستشفيات ولاية الخرطوم خلال الفترة من يناير الي أبريل 2010. بايجاز ، تم جمع 100 عينة (ن = 100) من مواقع مختلفة من غرف العمليات. و قد تم جمع العينات من مجمع العمليات الرئيسية في مستشفي الخرطوم بحري التعليمي و مستشفي الخرطوم التعليمي. عن طريق استخدام معيار البكتيريا المورفولوجية والتقنيات الكيميائية الحيوية ، سواء إيجابية الغرام وسلبية الغرام تم عزلها و تحديدها.

أكثر بكتيريا ملوثة لغرف العمليات تم عزلها هي العنقودية الذهبية (30.8%) ،العنقودية البشروية (20.5%) ، العنقودية الرمية (5.1%) ، الاشريكية القولونية (20.5%) ، والزائفة الزنجارية (15.4%) ، والمتقلبة الرائعة (5.1%) والامعائية المدرقية (2.6%).اعلي نسبة تلوث عزلت من أسرة (17.9%) ، الارضيات (15.4%) ،عربة الادوات (12.8%) ، الاحذية واجهزة التكييف (10.3%) ، المصاصات ، الكشافات ومكنات التخدير (7.7%) ، اسطوانات الأوكسجين والهواء ((5.1% .

كل العنقودية الذهبية التي عزلت من مستشفي الخرطوم بحري التعليمي مقاومة للمثسلين بنسبة 100% بينما 50% فقط مقاومة للمثسلين في مستشفي الخرطوم التعليمي. بالرغم من ذلك 50% من العنقودية الذهبية حساسة للإريثروميسين،جنتاميسين وكواموكسي كلاف في كل من مستشفي الخرطوم بحري التعليمي و مستشفي الخرطوم التعليمي.

العنقودية الرمية مقاومة للامبيسلين ولكن حساسة للإريثروميسين ، جنتاميسين وكواموكسي كلاف . كذلك العنقودية البشروية حساسة لجنتاميسين وكواموكسي كلاف ، الإ ان الامعائية المدرقية مقاومة للامبيسلين ،كواموكسي كلاف وحساسة للكلورامفينيكول . في مستشفي الخرطوم بحري التعليمي المتقلبة الرائعة حساسة لي للامبيسلين،كواموكسي كلاف ومقاومة للكلورامفينيكول ، بالمقابل المتقلبة الرائعة التي عزلت من مستشفي الخرطوم التعليمي مقاومة

للامبسيلين،جنتاميسين وكواموكسي كلاف، حساسة للكلورامفينيكول.الاشريكية القولونية التي عزلت من مستشفي الخرطوم التعليمي اكثر مقاومة للامبيسلين ، وكواموكسي كلاف وحساسة للكلورامفينيكول وجنتاميسين بينما التي عزلت من مستشفي الخرطوم بحري التعليمي 83.3% مقاومة للامبيسلين ، الكلورامفينيكول ، جنتاميسين و 66.7% حساسة كواموكسي كلاف .

100% من الزائفة الزنجارية عزلت من مستشفي الخرطوم بحري التعليمي مقاومة للكلورامفينيكول، 66.7% مقاومة للامبيسلين ،جنتاميسين وكواموكسي كلاف . بينما 100% الزائفة الزنجارية التي عزلت من مستشفي الخرطوم التعليمي مقاومة للامبيسلين و 66.7% مقاومة كواموكسي كلاف ، لكن حساسة للجنتاميسين و للكلورامفينيكول.

خلاصة البحث، ثبت ان هنالك انواع وانماط بكتيرية عديدة تسبب التلوث المستمر في غرف العمليات بمستشفي الخرطوم و الخرطوم بحري التعليمي.

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List of Abbreviations

AMP	Antimicrobial prophylaxis
$BaCl_2$	Barium Chloride
CFU	Colony Forming Unit
CRF	Coagulase Reacting Factor
DNA	Deoxyribonucleic acid
DW	Distilled Water
E. coli	*Escherichia coli*
E. cloacae	*Enterobacter cloacae*
ENT	Ear Nose and Throat
EPA	Environmental Protection Agency
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
H_2SO_4	Sulphuric acid
HEPA	High Efficiency Particulate Air
KIA	Kligler Iron Agar
KNTH	Khartoum North Teaching Hospital
KTH	Khartoum Teaching Hospital
MRSA	Methicillin Resistant *Staphylococcus aureus*
NCCLS	National Committee for Clinical Laboratory Standard
NNIS	National Nosocomial Infection Surveillance
OSHA	Occupational Safety and Health administration
P. aeruginosa	*Pseudomonas aeruginosa*
P. mirabilis	*Proteus mirabilis*
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
S.saprophyticus	*Staphylococcus saprophyticus*
SSI	Surgical Site Infections
VP	Voges-Proskauer

CHAPTER ONE

1. Introduction

1.1 Overview:

Nosocomial infections, also called “hospital acquired infections” can be defined as an infection acquired in hospital by a patient who was admitted for a reason other than that infection (Ducel *et al.*, 2002). An infection occurring in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility (Benenson, 1995). Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in healthcare settings are among the major causes of death and increased morbidity among hospitalized patients.

Bacterial contaminants of operating theatres are one of the most life –threatening source of nosocomial infection for patients. Surgical Site Infections (SSI) remains substantantial causes of morbidity and mortality among hospitalized patients and may be partially explained by the emergency of antimicrobial resistant pathogens and increase number of prosthetic implants and organs transplanted.

Hospital-acquired infections add to functional disability and emotional stress of the patient and in some cases, lead to disabling conditions that reduce the quality of life.

1.2 Rationale:

Nosocomial infections are also one of the leading causes of death and their economic costs are considerable. The increased lengths of stay for infected patients are the greatest contributor to cost prolonged stay, not only increases direct costs to patients or payers but also indirect costs due to lost work. The increased use of drugs, the need for isolation, and the use of additional laboratory and other diagnostic tests also contribute to costs.

Hospital-acquired infections add to the imbalance between resource allocation for primary and secondary healthcare by diverting scarce funds to the management of potentially preventable conditions. In addition, the advancing age of patients admitted to healthcare settings, the greater prevalence of chronic diseases among admitted patients, and the increased use of diagnostic and therapeutic procedures, all add to that problem (Ducel *et al*., 2002).

Contamination in operating theatres in Sudanese hospitals is not well studied; therefore, the aim of this study is to investigate the extent of contamination hoping for possible prevention and control in the near future.

1.3 Objectives:

1.3.1 General objective:

To isolate bacterial contaminants in operating theatres in Khartoum Teaching Hospital and Khartoum North Teaching Hospital.

1.3.2 Specific objectives:

1- To isolate and identify aerobic bacteria contaminants in operating theatres.

2- To identify the antibiotic susceptibility patterns of the isolated bacteria.

CHAPTER TWO

2. Literature Review

2.1 Definitions of nosocomial infections

Nosocomial infections, also called “hospital-acquired infections” can be defined as an infection acquired in hospital by a patient who was admitted for a reason other than that infection (Ducel *et al*., 2002). An infection occurring in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility (Benenson, 1995).

Nosocomial infections may also be considered either endemic or epidemic. Endemic infections are most common. Epidemic infections occur during outbreaks and are defined as an unusual increase above the baseline of a specific infection or infecting organism (Ducel *et al*., 2002).

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.

The infecting microorganisms are variable, depending on the type and location of surgery, and antimicrobials received by the patient. The main risk factor is the extent of contamination during the procedure (clean, clean contaminated, contaminated, dirty), which is, to a large part, dependent on the length of the operation, and the patient’s general condition (Report from NNIS System, 1991). Other factors include the quality of surgical technique, the presence of foreign bodies including drains, the virulence of the microorganisms, concomitant infection at other sites, the use of preoperative shaving, and the experience of the surgical team.

2.2 Most common nosocomial pathogens

2.2.1 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 compromised. 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).

2.2.2 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 bloodstream infections and are frequently resistant to antibiotics; beta-haemolytic streptococci are also important.
- Gram-negative bacteria: Enterobacteriacae (e.g. *E. 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, bacteriaemia, peritoneum infection). They may also be highly resistant.
- Gram-negative organisms such as Pseudomonas species are often isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients.
- 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 aerosol) (Ducel *et al.*, 2002).

2.3 Surgical Site Infection (SSI):

Before the 19th century, surgical patient's commonly developed post-operative "irritative fever," followed by purulent drainage from their incisions, overwhelming sepsis, and often death. It was not until the late 1860s, after Joseph Lister introduced the principles of antisepsis, when post-operative infectious morbidity decreased substantially.

Lister's work radically changed surgery from an activity associated with infection and death to a discipline that could eliminate suffering and prolong life. Based on National Nosocomial Infections Surveillance (NNIS) system, SSIs are the third most frequently reported nosocomial infection, accounting for 14% - 16% of all nosocomial infections among hospitalized patients. Among surgical patients, SSIs were the most common nosocomial infections, accounting for 38% of all such infections. Of these SSIs, two thirds were confined to the incision, and one third involved organs or spaces accessed during the operation. When surgical patients with nosocomial SSI died, 77% of the deaths were reported to be related to the infection, and the majorities (93%) were serious

infections involving organs or spaces accessed during the operation.

In 1980, Cruse estimated that an SSI increased a patient's hospital stay by approximately 10 days and cost (Cruse and Foord, 1980). The definition of SSI is mainly clinical: purulent discharge around the wound or the insertion site of the drain, or spreading cellulitis from the wound. Infections of the surgical wound (whether above or below the aponeurosis), and deep infections of organs or organ spaces are identified separately (Ducel *et al.*, 2002). Advances in infection control practices include improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis. Despite these activities, SSIs remain a substantial cause of morbidity and mortality among hospitalized patients. This may be partially explained by the emergence of antimicrobial-resistant pathogens and the increased numbers of surgical patients who are elderly and or have a wide variety of chronic, debilitating, or immunocompromising underlying diseases. There also are increased numbers of prosthetic implant and organ transplant operations performed. To reduce the risk of SSI, a systematic, but realistic approach must be applied with the awareness that this risk is influenced by characteristics of the patient, operation, personnel, and hospital (Alicia *et al*., 1999). According to data from the NNIS system, the distribution of pathogens isolated from SSIs has not changed markedly last decade, *S. aureus*, coagulase-negative staphylococci, Enterococcus species, and *E. coli* remain the most frequently isolated pathogens (Nooyen *et al.*, 1994). An increasing proportion of SSIs are caused by antimicrobial resistant pathogens, such as methicillin-resistant *S. aureus* (MRSA) (Schaberg *et al.*, 1991). In Sweden the design of many operating suites has been greatly influenced by the recommendations of the operating theatre of the Hygiene Subcommittee of the Medical Research Council in England (1962).

According to their recommendations an operating suite can be divided into four different zones:

A. Protective Zone: Including entrance lobby, recovery rooms and changing rooms.

B. Clean Zone: Including anaesthetic room, scrub-up room and inner lobby.

C. Sterile Zone: Including operating rooms, sterilizing rooms and lay-up rooms.

D. Disposal zone: Including sink room.

2.4 The sources of contamination:

2.4.1 Air

2.4.1.1 Airborne contamination and transmission:

Infection may be transmitted over short distances by large droplets and at longer distances by droplet nuclei generated by coughing and sneezing (Knight, 1973). Droplet nuclei remain airborne for long periods, may disseminate widely in an environment such as a hospital ward or an operating room, and can be acquired by (and infect) patients directly, or indirectly through contaminated medical device.

The number of organisms present in room air will depend on the number of people occupying the room, the amount of activity, and the rate of air exchange.

Bacteria recovered from air samples usually consist of Gram-positive cocci originating from the skin. They can reach large numbers if dispersed from an infected lesion, particularly an infected exfoliative skin lesion. However, since the contaminated skin scales are relatively heavy, they do not remain suspended in the air for long. Gram-negative bacteria usually found in the air only when associated with aerosols from contaminated fluids, and tend to die on drying (Ducel *et al*., 2002).

Wound infection caused by *S. aureus* acquired in the operating theatre may be prevented, in part, by improving the bacteriological quality of air during surgery. Total numbers of bacteria in an empty operating theatre should be < 35 colony forming units (CFU/m^3) with less than one colony *of Clostridium perfringens or S. aureus***.** During an operation, total air counts should be < 180 CFU/m^3 averaged over a 5-min period. These standards have never been validated scientifically, but represent a consensus view from investigations of outbreaks of post-operative wound infections, various studies carried out under experimental conditions, and the results were obtained during the commissioning of operating theatres (Report. Ventilation in operation suites,1972).

The major cause of deep post-operative wound sepsis is contamination from airborne CFUs, as well as contaminated equipment or gloves placed in the wound and direct inoculation from skin adjacent to the incision. The majority of airborne contamination originates from squamous skin from patients (Bethune *et al.*, 1965) and the surgical team (Blowers and McCluskey, 1965), with the concentration of CFUs in the operating theatre

shown to be proportional to the activity and numbers of people present. This is partly because of the control of other exogenous sources but also because a very low dose of bacterial inoculum is believed to be capable of initiating infection in some current procedures, notably the insertion of prosthetic implants. For example, in hip joint replacement, the deposition in the open wound of as few as 10 airborne particles carrying skin commensal bacteria of low pathogenicity is said to be associated with a rate of joint sepsis of 1% rising to over 5% when the dose exceeds 100 particles (Lidwell *et al*., 1982). Kaur and Hans (2007) reported that common bacterial contaminants in operating theatre from air *S. epidemidis* was 39 %, *S. aureus* was17.4%, *P. aeruginos* was 30% and coliform was 13%.

2.4.1.2 Factors influencing airborne contamination in operating theatres:

A. Type of surgery.

B. Quality of air provided.

C. Rate of air exchange.

D. Number of persons present in operating theatre.

E. Movement of operating room personnel.

Movement can shed up to 10,000 skin scales per minute, of which 10% carry clusters of microorganisms. These will contain *S. aureus* and coagulase-negative staphylococci which are frequent causes of infection of joint replacement. In a well-run and organized theatre, movement is kept to a minimum and dispersal of skin organisms are reduced.

F. Level of compliance with infection control practices.

G. Quality of staff clothing.

H. Quality of cleaning process (Ducel *et al*.,2002).

2.5 Environmental surfaces:

Environmental surfaces of operating rooms (e.g., tables, floor, walls, ceiling, and lights)

in the United States (U.S) 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 (Sessler *et al.*, 1992).

There are no data to support routine disinfection 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 (Nichols, 1992). This is in keeping with the Occupational Safety and Health Administration (OSHA) requirements that all equipment and environmental surfaces be cleaned and decontaminated after contact with blood or other potentially infectious materials. 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 (Rudnick *et al*., 1996).

There are no data to support special cleaning procedures or closing of an operating room after a contaminated or dirty operation has been performed (Laufman, 1986; Nichols, 1992). Tacky mats placed outside the entrance to an operating room have not been shown to reduce the number of organisms on shoes or stretcher wheels, nor do they reduce the risk of SSI (Laufman, 1986; Classen *et al*.,1992; Mayhall, 1993).

2.5.1 Floor:

Air borne 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 microorganisms which contaminate the floors of hospital wards are commonly assumed to be important sources of nosocomial infections. Attempts to reduce the level of contamination of floors by scrubbing or disinfection have not met with great success. For example, although cleaning reduced the bacterial numbers on a floor by over 80%, unless the areas were protected against recontamination,

the bacterial numbers returned to the precleaning level after about 1 hour (Ayliffe *et al*., 1966). The transfer of microorganisms, on the soles of footwear and trolley wheels, into clean areas of hospitals such as operating theatres is potentially a major source of recontamination of these areas and a variety of different decontaminating mats have been developed in attempts to control it (Meddick, 1977).

2.5.2 Operation 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 of contamination was due to sedimentation of airborne bacteria-carrying particles. The contamination of the wall was about half that of the floor and the 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)**.**

2.6 Shoes:

Pathogenic bacteria were found on all shoes, with outdoor shoes the most heavily contaminated. Floor bacteria may contribute up to 15% of airborne bacteria (CFU) in operation room; dedicated operation room shoes and strict floor washing protocols may control the level of shoe contamination, especially coagulase-negative staphylococci (Amirfeyz *et al.,* 2007). Coliform, bacilli, diptheroid and micrococci species were also isolated from theatre shoes. All of these have been demonstrated to result in lower limb arthroplasty infection (Nelson *et al*., 1973).

2.7 Suckers:

The tips of suckers have rates of contamination of 16% - 55%, but they are still used by most surgeons (Grabe *et al.,* 1985).

2.8 Gloves:

Gloves become contaminated during preparation of the skin of the patient and double glove is adviced (McCue *et al.,* 1981).

2.9 Equipments:

The contamination of some equipments commonly in use during arthroplasty, including the collection bag used during hip replacement and the 'sterile' handles attached to the theatre lights, has not been explored. The bags are often used as a resting place for instruments and swabs during surgery, a habit which may facilitate transfer of bacteria into the wound. Ensayef *et al* (2009) reported common bacterial contaminants in operating theatres during 2001 was *S. epidermidis*, followed by *Pseudomonas aeruginosa*, whereas in 2002 coliform bacteria were the highest followed by *P. aeruginosa*. The percentage of positive culture bacteria in 2001 were 39.1% for *S. epidermidis*, *P. aeruginosa* was 30.4%, *S. aureus* was17.4% and coliform was 13.0%. In 2002 *S. epidermidis* was 8.3%, *S. aureus* was 4.2%, *P. aeruginosa* was 25.0% and coliform was 62.5%.

2.10 Prevention and control of infection in operating theatres:

2.10.1 Ventilation:

In the 1960s Charnley introduced the concept of ultraclean air systems and suggested that the cemented prosthesis may contain a system uniquely sensitive to infection by very small bacterial inocula, which were derived from airborne particles. Fresh filtered air, appropriately circulated, will dilute and remove airborne bacterial contamination and also eliminates smells. Desirable ventilation rates, expressed in air changes per hour, vary with the purpose of a particular area (Ducel *et al*., 2002). High-risk hospital areas (operating rooms, nurseries, intensive care units, oncology, and burn units) should have air with minimal bacterial contamination. Adequate ventilation systems require proper design and maintenance to minimize microbial contamination. All outdoor air inlets must be located as high as possible above ground level; inlets must be remote from ventilation discharge outlets, incinerators, or boiler stacks. Within rooms, the location of air inlets and exhaust outlets influences the movement of air. High wall or ceiling inlets and low wall outlets allow clean air to move downward through the area toward the contaminated floor where it is removed through the low exhaust. This pattern is for a total area which patients

receive care, and areas subject to heavy contamination. Filters used in the ventilation systems must meet standards for the patient care activity of the area. High-efficiency filters must be provided in systems serving areas where patients are particularly susceptible to infection (Ducel *et al*., 2002).

Regular inspection and maintenance of filters, humidifiers, and grills in the ventilation system must be performed and documented. Cooling towers and humidifiers should be regularly inspected and cleaned to prevent aerosolization of Legionella species (Ducel *et al*., 2002).

Zoning of air systems may confine the air of a department to that department alone. A design that enables air pressure to control air movement into or out of a specific room or area will control the spread of contamination. Positive air pressure is recommended for areas which must be as clean as possible. It is achieved by supplying more air into an area than can be removed by the exhaust ventilation system. This produces an outflow around doors and other openings, and decrease entry of air from more contaminated areas. Negative air pressure is recommended for contaminated areas, and is required for isolation of patients with infections spread by the airborne route. It is achieved by supplying less air to theatre than can be removed by the ventilation system. Negative air pressure produces an inflow around openings and reduces the movement of contaminated air out of the area. For effective air pressurization all doors must be kept closed except for essential entrances and exits (Ducel *et al.*, 2002).

2.10.2 Ultra-clean air:

For minimizing airborne particles, air must be circulated into the room with a velocity of at least 0.25 m/sec through a high-efficiency particulate air (HEPA) filter, which excludes particulate matter of defined size. If particles 0.3 microns in diameter and larger are removed, the air entering the room will be essentially clean and free of bacterial contaminants. This principle has been applied to microbiology laboratories, pharmacies, special intensive care units, and operating rooms for operating theatres, a unidirectional clean airflow system with a minimum size of 9 m^2 (Ducel *et al*., 2002).

2.10.3 Sterilization:

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

2.10.3.1 Conventional sterilization of surgical instruments:

Inadequate sterilization of surgical instruments has resulted in SSI outbreaks (Sessler *et al*., 1992). Surgical instruments can be sterilized by steam under pressure, dry heat, ethyl oxide, or other approved methods. Microbial monitoring of steam autoclave performance is necessary and can be accomplished by use of a biological indicator.

2.10.3.2 Flash sterilization of surgical instruments:

The Association for the Advancement of Medical Instrumentation defines flash sterilization as "the process designated for the steam sterilization of patient care items for immediate use (Association for the Advancement of Medical Instrumentation, 1996). 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 (Favero and Bond, 1991). Flash sterilization is not recommended as a routine sterilization method because of the lack of timely biologic indicators to monitor performance, absence of protective packaging following sterilization, possibility for contamination of processed items during transportation to operating rooms, and use of minimal sterilization cycle parameters (i.e., time, temperature, pressure) (Favero and Manian, 1993). Many hospitals have placed equipment for flash sterilization in close proximity to operating rooms and new biologic indicators that provide results in 1 to 3 hours are now available for flash-sterilized items (Rutala *et al*., 1993).

2.10.4 Disinfection of patient equipment:

Disinfection removes microorganisms without complete sterilization to prevent transmission of organisms between patients (Rutala, 1996). It meets 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).

To be acceptable in the hospital environment, they must also be easy to use, non-volatile, not harmful to equipment, staff or patient, free from unpleasant smells, and effective within a relatively short time. Different products or processes achieve different levels of disinfection. These are classified as high, intermediate or low-level disinfection (Alvarado *et al*., 2000).

- **High-level disinfection** (critical) — this will destroy all microorganisms, with the exception of heavy contamination by bacterial spores.
- **Intermediate disinfection** (semi-critical) — this inactivates *Mycobacterium tuberculosis*, vegetative bacteria, most viruses and most fungi, but does not necessarily kill bacterial spores.
- **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. These levels of disinfection are attained by using the appropriate chemical product in the manner appropriate for the desired level of disinfection.

2.10.5 Antimicrobial prophylaxis:

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent that initiates just before an operation begins (Nichols and Holmes, 1995).

AMP is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intra-operative contamination to a level that cannot overwhelm host defenses. AMP does not pertain to prevention of SSI caused by post-operative contamination. Intravenous infusion is the mode of AMP delivery used most often in modern surgical practice (Nooyen *et al*., 1994). Essentially all confirmed AMP indications pertain to elective operations in which skin incisions are closed in the operating room. Four principles must be followed to maximize the benefits of AMP:

• Use an AMP agent for all operations or classes of operations in which its use has been shown to reduce SSI rates based on evidence from clinical trials or for those operations after which incisional or organ space SSI would represent a catastrophe.

• Use an AMP agent that is safe, inexpensive, and bactericidal *in vitro* spectrum that covers the most probable intra-operative contaminants for the operation.

• Time the infusion of the initial dose of antimicrobial agents so that a bactericidal concentration of the drug is established in serum and tissues by the time the skin is incised (Classen *et al*., 1992).

• Maintain therapeutic levels of the antimicrobial agent in both serum and tissues throughout the operation and until, at most, a few hours after the incision is closed in the operating room (Mayhall, 1993). Present in all surgical wounds, therapeutic serum levels of AMP agents are logically important in addition to therapeutic tissue levels. Fibrin-enmeshed bacteria may be resistant to phagocytosis or to contact with antimicrobial agents that diffuse from the wound space. AMP is sometimes indicated for operations that entail incisions through normal tissue and in which no viscous is entered and no inflammation or infection is encountered. Two well-recognized AMP indications for such clean operations are: (1) when any intravascular prosthetic material or a prosthetic joint will be inserted, and (2) for any operation in which an incisional or organ space SSI would pose catastrophic risk (Da Costa *et al.*, 1998).

2.10.6 Surgical attire:

The term surgical attire refers to scrub suits, caps, hoods, shoe covers, masks, gloves, and gowns. Although experimental data show that live microorganisms are shed from hair, exposed skin, and mucous membranes of operating room personnel, few controlled clinical studies have evaluated the relationship between the use of surgical attire and SSI risk (Hardin and Nichols, 1995). Nevertheless, the use of barriers seems prudent to minimize a patient's exposure to the skin, mucous membranes, or hair of surgical team members, as well as to protect surgical team members from exposure to blood and blood borne pathogens (e.g., human immunodeficiency virus and hepatitis viruses).

2.10.6 .1 Scrub suits:

Surgical team members often wear a uniform called “scrub suit” that consists of pants and a shirt. Policies for laundering, wearing, covering, and changing scrub suits vary greatly. Some policies restrict the laundering of scrub suits to the facility, while other facilities have policies that allow under laundering by employees. There are no well-controlled studies evaluating scrub suit laundering as an SSI risk factor (Copp *et al*., 1986). Some facilities have policies that restrict the wearing of scrub suits to the operating room, while other facilities allow the wearing of cover gowns over scrub suits when personnel leave the operation room. The Association of Operating Room Nurses (1999) recommends that scrub suits be changed after they become visibly soiled and that they be laundered only in an approved and monitored laundry facility.

Additionally, OSHA regulations require that “if a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible.”

2.10.6.2 Masks:

The wearing of surgical masks during operations to prevent potential microbial contamination of incisions is a long standing surgical tradition. Nevertheless, wearing a mask can protect the wearer’s nose and mouth from inadvertent exposures (i.e., splashes) to blood and other body fluids. OSHA regulations require that masks in combination with protective eye wear, such as goggles or glasses with solid shields, or chin-length face shields be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious material may be generated and eye, nose, or mouth contamination can be reasonably anticipated (Occupational Safety and Health Administration, U.S. Department of Labor, 1991).

2.10.6.3 Surgical caps, hoods and shoe covers:

Surgical caps, hoods are inexpensive and reduce contamination of the surgical field by organisms shed from the hair and scalp. SSI outbreaks have occasionally been traced to organisms isolated from the hair or scalp e.g *S. aureus* and group A Streptococcus even when caps were worn by personnel during the operation and in the operating suites (Mastro *et al*., 1990). The use of shoe covers has never been shown to decrease SSI risk or to decrease bacteria counts on the operating room floor (Humphreys *et al.*, 1991).

Shoe covers may, however, protect surgical team members from exposure to blood and other body fluids during an operation. OSHA regulations require that surgical caps or hoods and shoe covers or boots be worn in situations when gross contamination can reasonably be anticipated (e.g., orthopedic operations, penetrating trauma cases).

2.10.6.4 Sterile gloves:

Sterile gloves are put on after donning sterile gowns. A strong theoretical rationale supports the wearing of sterile gloves by all scrubbed members of the surgical team. Sterile gloves are worn to minimize transmission of microorganisms from the hands of team members to patients and to prevent contamination of team members ’hands with patients’ blood and body fluids. If the integrity of a glove is compromised (e.g punctured), should be changed as promptly as safety permits (Dodds *et al.*, 1988).

Wearing two pairs of gloves (double-gloving) has been shown to reduce hand contact with patients’ blood and body fluids when compared to wearing only a single pair (Short and Bell, 1993).

2.10.6.5 Gowns and drapes:

Sterile surgical gowns and drapes are used to create a barrier between the surgical field and potential sources of bacteria. Gowns are worn by all scrubbed surgical team members and drapes are placed over There are limited data that can be used to understand the relationship of gown or drape characteristics with SSI risk.

Gowns and drapes are classified as disposable (single use) or reusable (multiple uses). Regardless of the material used to manufacture gowns and drapes, these items should be impermeable to liquids and viruses. In general, only gowns reinforced with films, coatings, or membranes appear to meet standards developed by the American Society for Testing and Material. However, such “liquid-proof” gowns may be uncomfortable because they also inhibit heat loss and the evaporation of sweat from the wearer’s body. These factors should be considered when selecting gowns (Lewis and Brown, 1998).

CHAPTER THREE

3. Materials and Methods

3.1 Study approach:

Qualitative research approach.

3.2 Study design:

It is based on descriptive study.

3.3 Study area:

This study was conducted in KNTH and KTH.

3.4 Study duration:

This study was carried out during the period from January 2010 to April 2010.

3.5 Sample size:

One hundred (n=100) swabs were collected from certain sites in operating theatres, trollis, suckers, anesthesia machines, floors , shoes, oxygen cylinders, air conditions, focusing lamps, air settles and beds.

3.6 Methods:

3.6.1 Sample collection:

The specimens were collected from operation theatres (General surgery, ENT, Delivery) in each KNTH and KTH. The samples were collected by dipping sterile swabs in normal saline and then equipments of interest were swabbed gently under aseptic conditions to avoid contamination, the air samples were collected by used blood agar and MacConkey agar opened in air. The samples were transferred to the laboratory immediately for bacteriological examination.

3.6.1.1 Culture of specimens:

Under aseptic condition (near Bunsen burner), all collected specimens were inoculated on blood agar and MacConkey agar (Laboratories CONDA, Spain) and the inoculated plates were incubated aerobically at 37°C for 24 hours.

3.6.1.2 Examination of the growth:

Discrete colonies from primary cultures that showed significant growth were tested at the end of the incubation period for their fermentative and non-fermentative character on MacConkey agar, haemolysis on blood agar.

3.6.2 Primary identification:

3.6. 2.1 Gram stain:

A smear was prepared from each suspected isolate by dipping a portion of a colony in a slide containing normal saline and well mixed. The suspension was spread over the slide and left to dry. Then each dried smear was fixed by passing the slide through the flame of

Bunsen burner 2-3 times. Fixed smears were stained with Grams stains according to the well known conventional method (Cheesbrough, 1984). Smears were prepared from significant bacterial growth (colonies), stained with Gram stain method and examined under the microscope to detect the appearance of bacteria and stain reaction

3.6.3 Secondary identification:

3.6.3.1. Catalase test:

This test was used to differentiate catalase producing bacteria such as staphylococci, from non-catalase producing bacteria such as streptococci. This test was performed by using the tube method (Cheesbrough, 1984)

3.6.3.1.1 Principle:

The breakdown of hydrogen peroxide (H_2O_2), into oxygen and water is mediated by the enzyme catalase. When a small amount of an organism that produces catalase is introduced into H_2O_2, rapid elaboration of bubbles of oxygen, the gaseous product of the enzyme's activity is produced.

3.6.3.1.2 Procedure:

This test was performed by using tube method as described above. A sterile wooden stick was used to pick a portion of the test organism and immersed in 3% H_2O_2 solution; production of air bubbles indicates catalase positive reaction.

3.6.3.2 Coagulase test:

3.6.3.2.1 Principle:

This test is used to differentiate *S. aureus* which produces the enzyme coagulase from *S. epidermidis* and *S. saprophyticus* which don't produce coagulase. *S. aureus* produced two forms of coagulase, bound and free. Bound coagulase or clumping factor is bound to bacterial cell wall and reacts directly with fibrinogen. This results in an alteration of fibrinogen so that it precipitates on the staphylococcus cell, causing the cells to clump

when bacterial suspension is mixed with plasma. The presence of bound coagulase correlates well with free coagulase that causes the formation of a clot. When *S. aureus* colonies are incubated with plasma, the clotting mechanism involves activation of plasma coagulase–reacting factor (CRF), which is modified or derived thrombin molecule, to form coagulase CRF complex. This complex in turn reacts with fibrinogen to produce the fibrin clot (Baiely and Scott, 1998).

3.6.3.2.2 Procedure:

A clean slide was divided into two sides (test and control). A drop of distilled water (D.W.) was placed in the two sides. Then, using a sterile wire loop, 2-3 colonies from catalase producing bacteria were mixed with D.W. to form smooth milky suspension. Then a drop of undiluted plasma was added to test side only and the control side was left as negative, mixed with the suspension (rotated), reaction appeared as clumping, became visible to the naked eye within 10 seconds in the test side, while the control side remained as control negative.

3.6.3.3 DNase test:

3.6.3.3.1. Principle:

Deoxyribonuclease hydrolyzes deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNase–producing colonies are therefore surrounded by clear zone due to DNA hydrolysis (Cheesbrough, 1984).

3.6.3.3.2 Procedure:

By using sterile cotton tipped swab, sub-culture was done from the test organism by using line streaking on sterile dry surface medium containing DNA, incubated at 37°C for overnight. The second day, few drops of 1% hydrochloric acid was added to precipitate

unhydrolyzed DNA. The clear zones around the streaking line indicated positive test for DNase.

3.6.3.4 Mannitol fermentation:

This test was done to differentiate between species of staphylococci. Some species are able to ferment mannitol, others are not. The test was done by inoculating the bacteria on mannitol salt agar, and then incubated at 37ºC for 24 hours. Changes of phenol red indicator of the medium into yellow indicate mannitol fermentation (Cheesbrough, 1991).

3.6.3.5 Novobiocin disk:

It is used to differentiate *S. epidermidis* from *S. saprophyticus. S. epidermidis* is sensitive while *S. saprophyticus* is resistant (Cheesbrough, 1984).

3.6 .3.6 Oxidase test: (Cytochrome oxidase test):

3.6.3.6 .1 Principle:

The test is based on the production of oxidase enzyme that catalyzes the transport of electrons between electron donors in the bacteria and a redox dye-tetramethyl-p-phenylene-diamine in which the phenylene-diamine in the reagent is oxidized to indophenols blue with a deep purple color (Aslanzadeh, 2006).

3.6.3.6.2 Procedure:

A piece of filter paper impregnated with oxidase reagent was placed on a clean slide using sterile forceps. Then a wooden stick was used to pick a colony of test organism and placed on the filter paper; the development of a blue purple color was observed within few seconds. When blue-purple color was produced within 10 seconds, the test was considered positive (oxidase produced). When no blue-purple was produced within 10 seconds, the test was considered negative (no oxidase produced) (Cheesbrough, 1984).

3.6.3.7 Citrate utilization test:

3.6.3.7.1 Principle:

The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen. The test organism is cultured in a medium which contains sodium citrate, ammonium salts and the indicator bromo-thymol blue. Positive test is shown by growth in the medium and change in color of the indicator from green to blue, due to the alkaline reaction, following citrate utilization (Cheesbrough, 1984).

3.6.3.7.2 Procedure:

A well isolated colony from a pure test isolate was picked using straight wire loop and inoculated as a single streak on the slant surface of the Simmon's citrate agar tube. The tube was incubated at 37ºC for 24-48 hours and observed daily for 7 days. Visible colonial growth along the inoculums, with deep blue color was considered positive (citrate utilized). When no colonial growth along the inoculums and no color change, the result was considered negative (citrate not utilized).

3.6.3.8. Kligler's iron agar (KIA):

3.6.3.8.1 Principle:

This test is used to detect the ability of test organism to ferment glucose (0.1%) and lactose (1.0%) producing acid only or acid and gas, and its ability to produce hydrogen sulphide (H_2S), when cultured on butt and slant of KIA medium (Cheesbrough,1984). The acid produced by the bacterium changes the pH of the medium to acidic and turns color to yellow due to presence of phenol red indicator. Acid production limited to only the butt of the tube is indicative of glucose utilization. This is because the concentration of glucose is lower than the lactose, thus the acid production is not very extensive. Acid production in the slant and the butt indicates lactose fermentation, because of the relatively high concentration of this sugar, thus leading to extensive acid production (Aslanzadeh, 2006). Gas is produced by aerogenic bacteria appeared as cracks in the medium as well as an air

gap at the bottom of the test tubes (Aslanzadeh, 2006). H_2S is produced when sulphur-containing amino acids (sodium thiosulphate) are decomposed to H_2S. H_2S is reacted with the ferric citrate indicator present in the medium to form ferric sulphide which is a black insoluble compound (Aslanzadeh, 2006).

3.6.3.8.2 Procedure:

A well isolated test colony was removed from the agar plate using a straight wire and stabbed into the bottom of the KIA tube. When the inoculated wire was removed from the bottom of the tube, the slant surface was streaked. The inoculated tube was then incubated at 35ºC for 18-24 hours (Aslanzadeh, 2006). When there was no carbohydrate fermented, the alkaline slant /alkaline butt showed red-red color. This was a characteristic of non-fermentative bacteria, such as *P. aeruginosa*. When glucose was fermented, and lactose was not fermented, the alkaline slant /acidic butt gave red-yellow color. This was characteristic of non-lactose-fermenting bacteria, such as shigella species (Aslanzadeh, 2006). When glucose was fermented, lactose was not fermented, H_2S was produced, the alkaline slant/acidic butt showed red-black color. This was characteristic of non-lactose fermenting bacteria, such as salmonella species and proteus species .When both glucose and lactose were fermented, the acidic slant/acidic butt gave yellow-yellow color. This was characteristic of lactose–fermenting coliforms, such as *E. coli*.

3.6.3.9 Urease test:

3.6.3.9.1 Principle:

The test organism is cultured in a medium which contains urea and the indicator phenol red. If the strain is urease-producer, the urea in the medium is broken down by hydrolysis to give ammonia and carbon dioxide. The ammonia changes the medium to alkaline which is indicated by the color change to red-pink (Cheesbrough, 1984).

3.6.3.9. Procedure:

The surface of the Christensen urea agar was streaked with the test organism using wire loop, and then incubated at 35ºC for 18-24 hours. When the color of the inoculated medium was red-pink, the test was considered positive (urease produced). When there

was no color change, the test was considered negative (no urease produced) (Cheesbrough, 1984).

3.6.3.10. Indole test:

3.6.3.10.1 Principle:

The test organism is cultured in a medium which contains tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with production of indole, pyruvic acid and ammonia. Indole produced is detected by Kovac's reagent or Ehrlich's reagent which contains 4-(P)-dimethyl aminobenzaldehyde that react with indole to produce a red color compound (Aslanzadeh, 2006).

3.6.3.10.2 Procedure:

Peptone water was inoculated with the test organism using wire loop and incubated at 35 ^{o}C for 18-24 hours. After the incubation period, Kovac's reagent was added down to the inner wall of the tube. When bright red color was produced at the surface of the medium, the test was considered positive (indole produced). When no bright red color was produced at the surface of the medium, the test was considered negative (no indole produced).

3.6.3.11 Methyl red test:

3.6.3.11.1 Principle:

This test is employed to detect the production of sufficient acid during fermentation of glucose and maintenance of conditions such that pH of an old culture is sustained below a value of about 4.5, as shown change in the color of the methyl red indicator which is added at the end period of incubation (Cheesbrough, 1984).

3.6.3.11.2 Procedure:

Peptone water was inoculated with the test organism using wire loop and incubated at 37 ^{o}C for 48 hours. After the incubation period, methyl red was added and well mixed. The

test is considered positive when bright red color was produced and negative when yellow color was produced.

3.6.3.12 Voges - Proskauer (VP) test:

3.6.3.12.1 Principle:

VP test detects butylene glycol producers. Acetyl-methyl carbinol (a cetoin) is an intermediate in the production of butylene glycol. In this test two reagents, 40% KOH and alpha-naphthol are added to the test broth after incubation and exposed to atmospheric oxygen. If a cetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha naphthol to produce red color. Role of alpha-naphthol is that of a catalyst and a color intensifier (Cheesbrough, 1991).

3.6.3.12.2 Procedure:

Tested organism is inoculated into glucose phosphate broth and incubated for at least 48 hours. 0.6 ml of alpha-naphthol is added to the test broth and shaken. 0.2 ml of 40% KOH is added to the broth and shaken. The tube was allowed to stand for 15 minutes. Appearance of red color is taken as a positive test. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents.

3.6.3 .13 Motility test:

This test detects motile organisms. The concentration of agar in a test medium is 0.4% or less to allow free spread of organisms. The test organisms were inoculated in a test medium by using a sterile straight loop, and then incubated at 37°C for 24 hours. Movement from stab line or hazy appearance throughout the medium indicates motile organism (Arora and Arora, 2007).

3.6.3.14 Antimicrobial sensitivity test:

Sensitivity testing was started with preparing McFarland standard as follow: briefly, 1% (v/v) solution of sulphuric acid (H_2SO_4) was prepared by adding 1ml of concentrated

H_2SO_4 to 99 ml distilled water, and mixed well. Then 1.17% (w/v) solution of Barium Chloride ($BaCl_2$) was prepared by dissolving 2.3 g of dehydrate $BaCl_2$ in distilled water. Then, 0.5 ml $BaCl_2$ solution was added to 99.5 ml 1% H_2SO_4, and mixed. A small volume of the turbid solution was transferred to a screw-cap bottle (Cheesbrough, 1991).

Kirby-Bauer diffusion disc method was performed according to (Cheesbrough, 1991) as follow: briefly, by using sterile wire loop several colonies of similar appearance were emulsified in 3 ml of sterile normal saline. The turbidity of the suspension was matched to that of McFarland standard.

Within 15 minutes after adjusting the turbidity of the inoculum suspension to that of the standard, a sterile cotton swab was dipped into the suspension and excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. The swab was then streaked over the surface of Muller Hinton agar and left to dry at room temperature (Cheesbrough, 1991).

By using sterile forceps antimicrobial discs were placed on the surface of agar, antimicrobial discs were placed on the surface of agar include ampicillin 10 mcg, amoxicillin and clavulanic acid 30 mcg, erythromycin 15 mcg, gentamicin10 mcg, methicilin 5 mcg and chloramphenicol 30 mcg. After placement, the discs were pressed on the surface of the medium to provide uniform contact. The plates were incubated at 37°C aerobically for overnight. By using a ruler, the diameter of each zone of complete growth inhibition around each of the disc were measured, the result of inhibition zone of test organism of each antibiotic was reported as susceptible, resistance, intermediate according to the National Committee for Clinical Laboratory Standard (NCCLS), to control result of inhibition zone of test organism control strain must be used.

3.7 Statistical analysis:

Data were analyzed by using Statistical Package for Social Sciences (SPSS-s). It was analyzed by descriptive statistics of frequency.

CHAPTER FOUR

4. Results

4.1 Morphological and Biochemical characteristics of isolated bacteria:

The results in table 4.1 illustrate the different genera and species of bacteria isolated from the operating theatres of KTH and KNTH. While 56.4% of the isolated bacteria were Gram-positive cocci, 43.6 % were Gram-negative bacilli.

Table 4.2 summarizes the biochemical characteristics of Gram-positive bacteria, whereas table 4.3 summarizes the biochemical properties of various Gram-negative bacteria. The results obtained in table 4.2 clearly identified the different species of staphylococci isolated from surgical theatres. Similarly, different genera of Gram-negative bacilli isolated were clearly identified in table 4.3.

4.2 Identification of isolated bacteria:

The incidence of positive cultures was 39/100 cultures (39%). Table 4.4 explains identification of isolated bacteria from operating theatres, which revealed that *S. aureus* was the most common isolate (30.8%), followed by coagulase negative Staphylococci (25.6%), *E. coli* (20.5%), *P. aeruginosa* (15.4%), *P. mirabilis* (5.1%), and *E. cloacae* (2.6%).

Table 4.5 summarizes the types of bacteria isolated from different sites of operation theatres. The types of bacteria isolated from operation theatre beds were 28.6% each of *E. coli*, *S. aureus*, *P. aeruginosa* and 14.3% *S. saprophyticus.* The isolated bacteria from floors were 33.3% each of *E. coli*, S. *aureus* and *S. epidermidis.* Bacteria isolated from shoes were 25% each of *S. aureus* and *P. aeruginosa* and 50% *S. epidermidis.* The isolated bacteria from trollis were 40% each of *E. coli* and *S. aureus* and 20% of *E. cloacae.* Bacteria isolated from air conditions were 20 % *P. aeruginosa* and 10% each of *S. aureus and P. mirabilis.* The types of bacteria isolated from focusing lamps were *E. coli* 33.3% and *S. epidermidis* 66.7%. Anaesthesia machines contained 66.7% *S. aureus* and 33.3% *S. epidermidis.* However, the type of bacteria isolated from oxygen cylinders was *S. epidermidis* 100%. Air within operating theatres contained 100% *S. aureus*, and bacteria isolated from suckers were 33.3 % each of *E. coli*, *P. aeruginosa* and *P. mirabilis.*

The highest contaminants were isolated from the beds (17.9%), followed by floors (15.4%), trollis (12.8%), shoes and air conditions (10.3%), suckers, focusing lamps and anaesthesia machines (7.7%), oxygen cylinders and air (5.1%). Table 4.6 frequency of bacteria isolated from different specialized surgical theatres of KTH and KNTH. The highly contaminated theatres are the general surgery theatres in KTH (28.3%), followed by general surgery theatres of KNTH (25.6%) and the delivery theatres of KNTH (17.9%) followed by, ENT theatre in KNTH which showed 12.8% contamination, followed by (7.8%) contamination in KTH delivery theatres and ENT theatres.

4.3 Antimicrobial sensitivity test:

Table 4.7 demonstrates the antimicrobial sensitivity of all bacteria isolated from the surgical theatres of KNTH and KTH.

While all isolated *S. aureus* (100%) from KNTH were methicillin resistant, only 50% were methicillin resistant in KTH. However, 50% of *S. aureus* isolated from KTH and KNTH were sensitive to erythromycin, gentamicin and co-amoxiclav.

S. saprophyticus was resistant to ampicillin but sensitive to erythromycin, gentamicin and co-amoxiclav; however *E. cloacae* was resistant to ampicillin and co-amoxiclav and sensitive to chloramphenicol.

In KNTH *P. mirabilis* was sensitive to ampicillin and co-amoxiclav and resistant to chloramphenicol. In contrast, *P. mirabilis* in KTH was resistant to ampicillin, co-amoxiclav and gentamicin, but sensitive to chloramphenicol.

E. coli isolated from KTH was highly resistant to ampicillin, gentamicin and chloramphenicol, while that isolated from KNT Hospital was sensitive to chloramphenicol and gentamicin. While 100% of *P. aeruginosa* isolated from KNTH were resistant to chloramphenicol, 66.7% of them were resistant to ampicillin, co-amoxiclav and gentamicin. However, 100% of *P. aeruginosa* isolated from KTH were resistant to ampicillin and 66.7% of them were resistant to co-amoxiclav, but sensitive to gentamicin and chloramphenicol.

Table 4.1: Bacterial morphology and Gram staining:

Staining	Number	Percentage
Gram- positive cocci	22	56.4%
Gram -negative bacilli	17	43.6 %
Total	39	100%

Table 4.2: Biochemical characteristics of isolated Gram- positive cocci:

Suspected organism	Catalase	Coagulase	DNase	Mannitol fermentation	Novobiocin disc
S. aureus	+	+	+	+	Sensitive
S. epidermidis	+	-	-	Weak	Sensitive
S. saprophyticus	+	-	-	+	Resistance

Kligler lron Agar				Biochemical properties							Identified bacteria
Slop	**Butt**	**Gas**	**H_2S**	**Oxidase**	**Indole**	**Motility**	**Methyl red**	**Voges- Proskauer**	**Citrate**	**Urea**	
Y	Y	+	-	-	+	+	+	-	-	-	***E. coli***
R	R	-	-	+	+	+	-	-	+	-	***P. aeruginosa***
R	Y	+	+	-	-	+	-	+	+	+	***P. mirabilis***
Y	Y	+	-	-	-	+	-	+	+	-	***E. cloacae***

Table 4.3: Biochemical characteristics of isolated Gram- negative bacilli**:**

Key: R: red, Y: yellow.

Table 4.4: Number and percentage of bacterial species isolated from operating theatres:

Bactreria	**No of isolate**	**Percentage%**
S. aureus	12	30.8
Coagulase negative Staphylococci	10	25.6
E. coli	8	20.5
P. aeruginosa	6	15.4
P. mirabilis	2	5.1
E. cloacae	1	2.6
Total	39	100

Operating theatre sites	Bacteria	No. of bacterial isolates	Percentage %
Beds	*S. aureus*	2	28.6
	E. coli	2	28.6
	P. aeruginosa	2	28.6
	S. saprophyticus	1	14.3
Floors	*E. coli*	2	33.3
	S. aureus	2	33.3
	S. epidermidis	2	33.3
Shoes	*S. aureus*	1	25
	S. epidermidis	2	50
	P. aeruginosa	1	25
Trolleis	*E. coli*	2	40
	S. aureus	2	40
	E. cloacae	1	20
Suckers	*E. coli*	1	33.3
	P. aeruginosa	1	33.3
	P. mirabilis	1	33.3
Air conditions	*S. aureus*	1	25
	P. aeruginosa	2	50
	P. mirabilis	1	25
Focusing lamps	*E. coli*	1	33.3
	S. epidermidis	2	66.7
Oxygen cylinders	*S. epidermidis*	2	100
Anesthesia machines	*S. aureus*	2	66.7
	S.	1	33.3

Table 4.5 The types of bacteria isolated from different operation theatres

Table 4.6 Frequency of bacteria isolated from the surgical theatres of Khartoum Teaching Hospital

.and Khartoum North Teaching Hospital

	Frequency of								
Operating theatres	No growth	*E. coli*	*S.aureus*	*P.aeruginosa*	*S.saprophyticus*	*S.epidermidis*	*E.cloacae*	*P.mirabilis*	Total
Khartoum North general surgery	20	5	2	2	N.G	1	N.G	N.G	30
Khartoum North delivery	3	1	2	N.G	N.G	3	1	N.G	10
Khartoum North Ear, Nose and Throat	5	N.G	2	1	1	N.G	N.G	1	10
Khartoum general surgery	19	2	5	1	N.G	2	N.G	1	30
Khartoum Delivery	7	N.G	1	1	N.G	1	N.G	N.G	10
Khartoum Ear, Nose and Throat	7	N.G	N.G	1	N.G	2	N.G	N.G	10
Total	61	8	12	6	1	9	1	2	100

Key N.G: No Growth

Table 4.7: Antimicrobial sensitivity of bacteria isolated from operating theatres Khartoum Teaching Hospital and Khartoum North Teaching Hospital.

		E. coli		*S. aureus*		*P. aeurginosa*		*S. saprophyticus*		*S. epidermidis*		*E. cloacae*		*P. mirabilis*	
Area	**Antibiotics**	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Khartoum North	Methicillin	N.T	N.T	100%	0%	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T
	Ampicillin	83.3%	16.7%	100%	0%	66.7%	33.3%	100%	0%	0%	100%	100%	0%	0%	100%
	Erythromycin	N.T	N.T	50%	50%	N.T	N.T	0%	100%	25%	75%	N.T	N.T	N.T	N.T
	Gentamicin	83.3%	16.7%	50%	50%	66.7%	33.3%	0%	100%	0%	100%	0%	100%	100%	0%
	Co- amoxiclav	33.3%	66.7%	50%	50%	66.7%	33.3%	0%	100%	0%	100%	100%	0%	0%	100%
	Chloramphenicol	83.3%	16.7%	N.T	N.T	100%	0%	N.T	N.T	N.T	N.T	0%	100%	100%	0%
Khartoum	Methicillin	N.T	N.T	50%	50%	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T
	Ampicillin	100%	0%	50%	50%	100%	0%	N.T	N.T	80%	20%	N.T	N.T	100%	0%
	Erythromycin	N.T	N.T	50%	50%	N.T	N.T	N.T	N.T	80%	20%	N.T	N.T	N.T	N.T
	Gentamicin	0%	100%	50%	50%	33.3%	66.7%	N.T	N.T	0%	100%	N.T	N.T	100%	0%
	Co- amoxiclav	100%	0%	50%	50%	66.7%	33.3%	N.T	N.T	0%	100%	N.T	N.T	100%	0%
	Chloramphenicol	0%	100%	N.T	N.T	33.3%	66.7%	N.T	N.T	N.T	N.T	N.T	N.T	0%	100%

Key S:sensitive R:resistance. N.T: Not tested. Co- amoxiclav: amoxicillin and clavulanic acid

CHAPTER FIVE

5. DISCUSSION

Assumingly, the operating theatres in any hospital should be situated in a strictly sterile zone. Therefore, and obviously, bacterial contamination of these surgical theatres is highly dangerous to the patients and performed huge hazard to the healthcare authorities. The associated structures of the operation theatres are uniquely predisposed to contamination by various microorganisms mainly bacteria, viruses, fungi and parasites. The results were in disagreement with those obtained by Ensayef *e*t *al.*,(2009) who reported that common bacterial contaminants in operating theatres in Al Imam Ali Hospital in Baghdad, during 2001 was *S. epidermidis* as Gram-positive bacteria and *P. aeurginosa* as Gram-negative bacilli, The prevalence of *S. epidermidis* 39.1% , *P. aeruginosa* 30.4% and coliform 13.0% and rate of positive cultures was 3.7% . The recent study of Ensayef *et al.*, (2009) in Baghdad reported common bacterial contaminants in operating theatres 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 klebsella 2.1%. The results were in disagreement with those obtained by BioInfo Bank Institute (2010) who reported the common bacterial contaminants in operating theatres 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 were fungi; the rate of positive culture was 19.1%.

In this study, were able to isolate *E. cloacae*, 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 differences.

The common contaminants were *S. aureus, S. epidermidis, P. aeruginosa and E. coli*. The contamination by *S. aureus* was usually exogenous from surgical staff (30% *S. aureus* as nasal carrier) and from air.

In case of *S. epidermidis* the possible contamination source was usually endogenous from normal skin flora of patients or exogenous from surgical staff. 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 theatres (Bellido and Hancock, 1993).

In case of *E. coli*, the possible source of contamination is the bowels of patient's normal flora or from urinary tract infections (McNicholas *et al*., 1991).

The rate of contamination in operating theatres was high in the beds (17.9%), followed by floors (15.4%), trollis (12.8%), shoes and air conditions (10.3%), suckers, focusing lamps and anaesthesia machines (7.7%), oxygen cylinders and air (5.5%). 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 contaminants. Comparing the antibiotic sensitivity of Gram-positive cocci and Gram-negative bacilli isolated, it was found that Gram-negative bacilli were more resistant than Gram- positive cocci. Most of *S. aureus* and enteric Gram-negative rods, such as, *E. coli*, and *P. aeruginosa*, were antibiotic resistant. This could be due to the wide spread antibiotics that routinely used in hospitals. Furthermore, bacterial strains in hospitals are often resistant to multiple antibiotics. This resistance is usually due to acquisitions of plasmids carrying several genes that encode the enzymes that mediate resistance (Levinson, 2004). The detection of different resistant bacterial isolates from different surgical sites in various hospitals is a living evidence that various serotypes of bacterial species are persistant contaminants of the operating theatres in Khartoum hospitals.

Conclusion

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

S. aureus, *S. epidermidis*, *E. coli* and *P. aeruginsoa* were found to be the most contaminating bacteria in operating theatres and these act as a dangerous source of nosocomial infection and life threatening 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 infections.

The highest bacterial contamination was found in beds, floors, trollis, shoes, air conditions and suckers. The isolated Gram-negative bacilli were more resistant to antibiotic than Gram-positive cocci.

Recommendations

1. The healthcare authorities should draw attention to this high prevalence of resistant bacterial contaminants in different sites of operating theatres of the main teaching hospitals in the capital of Khartoum.

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

3. Implementation of comprehensive infection control programs and surveillance of infections, 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.

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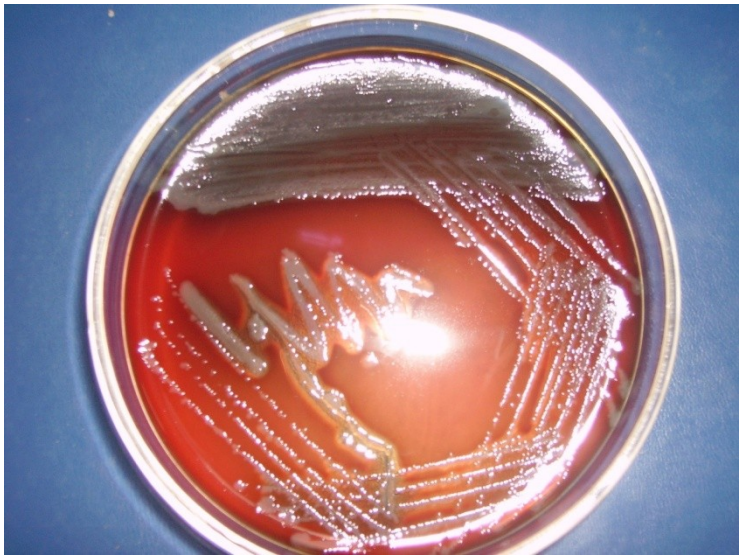
Appendix

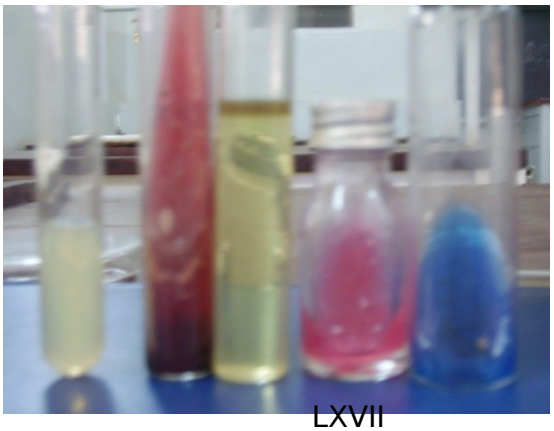
S. aureus in blood agar



P. aeruginosa in blood agar

Biochemical set of *P. aeruginosa*

Biochemical set of *P. mirabilis*



Biochemical set of *E. coli*

Media and Reagents

70% Alcohol

Blood agar

D.W.

Hydrogen peroxide

Iodine

Crystal violet

K.I.A.

Kovac's reagent

MacConkey agar

Mannitol salt agar

McFarland standard

Methyl red

Muller Hinton agar

Oxidase reagent

Peptone water

Physiological saline

Safranin

Hydrochloric acid

Semi solid media

Simmon's citrate agar

Urea agar